THE BEHAVIOR AND IMPACT OF ORGANIC ARSENICAL HERBICIDES IN THE FOREST: FINAL REPORT ON COOPERATIVE STUDIES

Logan A. Norris, Study Coordinator
USDA, Forest Service
Pacific Northwest Forest and Range Experiment Station
Corvallis, Oregon

Investigators:

Dr. Jack Allard
Dr. Walter B. Bollen
Mr. Paul R. Canutt
Dr. Robert Claeys
Dr. John O. Dickinson
Mr. Jim Evans
Mr. Jerry Exon
Mr. Robert Greaves
Dr. James R. Harr
Mr. Harry Hartwell
Mr. John Hughes
Dr. Loren Koller
Mr. Jack Lamberton
Mr. John Lyman
Dr. Hugh C. Maycumber
Mr. John Newman
Dr. Michael Newton
Dr. Logan A. Norris
Mr. Thomas Schroedel
Mr. Robert F. Tarrant
Dr. Sheldon Wagner
Dr. Paul Weswig
Mr. Yousif Yonan

Cooperators:

USDA, Forest Service
Region 1
Region 6
PNW For. & Range Exp. Stn.

US Dept. Interior
Bureau of Land Management
Bureau of Sport Fish. & Wildlife

State of Washington

State of Oregon

Dr. Hugh Maycumber, DVM

Key Chemical Company

Ansul Company

April 15, 1974

THE BEHAVIOR AND IMPACT OF ORGANIC ARSENICAL HERBICIDES IN THE FOREST: FINAL REPORT ON COOPERATIVE STUDIES

Logan A. Norris, Study Coordinator
USDA, Forest Service
Pacific Northwest Forest and Range Experiment Station
Corvallis, Oregon

Investigators:

Dr. Jack Allard
Dr. Walter B. Bollen
Mr. Paul R. Canutt
Dr. Robert Claeys
Dr. John O. Dickinson
Mr. Jim Evans
Mr. Jerry Exon
Mr. Robert Greaves
Dr. James R. Harr
Mr. Harry Hartwell
Mr. John Hughes
Dr. Loren Koller
Mr. Jack Lamberton
Mr. John Lyman
Dr. Hugh C. Maycumber
Mr. John Newman
Dr. Michael Newton
Dr. Logan A. Norris
Mr. Thomas Schroedel
Mr. Robert F. Tarrant
Dr. Sheldon Wagner
Dr. Paul Weswig
Mr. Yousif Yonan

Cooperators:

USDA, Forest Service
Region 1
Region 6
PNW For. & Range Exp. Stn.

US Dept. Interior
Bureau of Land Management
Bureau of Sport Fish. & Wildlife

State of Washington

State of Oregon

Dr. Hugh Maycumber, DVM

Key Chemical Company

Ansul Company

April 15, 1974

TABLE OF CONTENTS

TABLE OF CONTENTS

BEHAVIOR AND IMPACT OF ORGANIC ARSENICAL HERBICIDES IN THE FOREST : FINAL REPORT ON COOPERATIVE STUDIES

INTRODUCTION

The organic arsenic-containing herbicides cacodylic acid (dimethylarsinic acid) and MSMA (monosodium methanearsonate) are used as precommercial thinning agents in Pacific Northwest forests.

These chemicals are highly effective tools for thinning overstocked young stands of conifers. Many thousand acres of forest land in the Pacific Northwest would benefit from such treatment.

In 1969, the Okanogan National Forest reported the death of eight range cattle in forest areas treated with organic arsenic compounds for precommercial thinning. Uncertainty about the source of the arsenic and the significance of arsenic residues in hair and tissue from four of the cattle prompted the Regional Foresters of the Regions 1 and 6 to notify all Forests to immediately discontinue the use of MSMA and cacodylic acid for thinning.

Interested scientists and land managers from several organizations in the Pacific Northwest met to determine if gaps existed in our knowledge about the hazards associated with the use of organic arsenic herbicides in the forest. A series of cooperative studies to determine the behavior and impact of these compounds in the forest was initiated as a result of these meetings. A list of cooperators and cooperating agencies is in the appendix.

The studies were specifically designed to:

1. Measure the exposure of applicators to organic arsenic compounds and evalute the hazards of this exposure.
2. Measure the initial distribution of organic arsenic compounds among components of the forest environment.
3. Determine the persistence, movement, and fate of organic arsenic compounds in the forest.
4. Characterize the acute and chronic toxicity of these chemicals to large and small animals.

The results of these studies and other pertinent information are used to determine the degree of risk involved in current chemical thinning practices and to form a basis for making recommendations for future practices to minimize these risks.

This report is divided into two major parts. The first part reports the results of the cooperative studies in each of the following three major areas: (1) human exposure and health, (2) behavior of arsenicals in the forest, and (3) impact of arsenicals on domestic and wild animals. The second part deals with operational aspects of safe precommercial thinning with arsenicals.

RESULTS OF COOPERATIVE STUDIES

EXPOSURE AND EFFECTS ON HUMAN HEALTH

Five studies were conducted which deal with applicator exposure to arsenicals and their impact on human health. Tarrant and Allard monitored arsenic in urine from applicators during the summer of 1969 to obtain a measure of applicator exposure to the chemical. Allard did work of a similar nature during the summer of 1970, but added considerable emphasis to determining the impact of exposure to the arsenicals on human health. In 1971, Wagner measured arsenic levels in blood and 24-hour urine samples. Norris studied arsenic in urine at different times during a work week in an effort to find the best time to collect urine for monitoring applicator exposure during operational thinning. In 1972, Norris looked for a relationship between the concentration of arsenic in a single urine sample and the amount of arsenic voided in a 24-hour sample; so single urine samples could be used in operational thinning to predict total arsenic excretion in 24-hours.

Arsenic Levels in Urine From Forest Workers Applying Silvicides

R. F. Tarrant
Pacific Northwest Forest and Range Experiment Station
Portland, Oregon

and

Jack Allard
Washington State Department of Social And Health Services
Wenatchee, Washington

This study was conducted during the 1969 thinning season to determine the exposure of forest workers to arsenical silvicides based on their urine arsenic levels. The results have been published in the Archives of Environmental Health 24:277-280 (Appendix 2).

Tarrant and Allard asked the following questions:

1. What is the concentration of arsenic in urine collected from applicators on Monday and Friday of each work week?
2. Does this concentration vary with the number of weeks worked, the arsenical applied, or the method of application?

The workers wore cotton workpants and shirts. Cotton work gloves were laundered daily. Details of the study are in Appendix 1. In summary, the exposed workers had a significantly higher level of arsenic in urine than the control group. Maximum residues were observed in samples collected on Friday, but in most cases arsenic levels were near normal the following Monday. The concentration of arsenic on a given day of the week did not increase during the thinning season which indicates no rapid accumulation of high quantities of arsenic by exposed workers. Approximately 46% of the urine samples collected from exposed workers on Fridays contained concentrations of arsenic in excess of 0.3 ppm. In contrast, only 4% of workers exceeded this concentration on Mondays. Neither the chemical nor the method of application had a significant effect on urine arsenic levels. This report clearly established applicators are exposed to the chemical and urine arsenic levels are an important index to level of exposure. Further, many workers exceed the maximum suggested level of arsenic in urine(0.3ppm) but even brief periods of no exposure result in a rapid drop in arsenic excretion.

Arsenical Silvicide Effects on Human Health

Jack Allard
Washington State Department of Social and Health Services
Wenatchee, Washington

This study was conducted during the 1970 thinning season to determine concentration of arsenic in urine and changes in body chemistry of applicators after season-long exposure to the arsenical silvicides. The study considered the potential for using arsenic levels in urine or changes in body biochemistry as an index to the significance of chronic exposure to arsenic.

The study asked the following questions:

1. What is the concentrations of arsenic in urine from applicators of arsenical silvicides, and how does the concentration change with the increasing length of exposure?
2. What changes in blood and kidney functions are detected in applicators of arsenical silvicides following chronic exposure?
3. What changes occur in the patterns of illness of applicators during the thinning season?

Applicators were instructed in handling techniques to minimize personal exposure. Skin cream, goggles, and gloves were prescribed. The results of urine-arsenic analysis for five groups of unexposed and exposed forest workers are in Table 1.

The disastrous 1970 fire season in eastern Washington prevented collection of data for this study on the planned schedule. The data that is available show average concentrations of arsenic in urine were lower than in Tarrant and Allard's 1969 study where 46% of Friday urine arsenic levels exceeded 0.3 ppm.

In 1970, Allard found the highest concentrations of arsenic in urine from applicators using cacodylic acid. Tarrant and Allard's 1969 data suggest workers applying cacodylic acid also had a higher concentration of arsenic in their urine than workers applying MSMA, but this difference was not statistically significant.

It is the opinion of those supervising the 1969 and 1970 applicators included in the two studies that the 1970 work crews received more comprehensive and intensive training in handling and applying the silvicide. This training, rather than the addition of goggles, may be primarily responsible for the reduction in urine-arsenic levels in the 1970 study.

A wide variety of clinical laboratory data on exposed and controlled workers were collected at the beginning and end of the thinning season (Table 2a, b and c).

TABLE 1. ARSENIC IN URINE FROM FOREST WORKERS USING MSMA OR CACODYLIC ACID, FOREST SERVICE, 1970.

	ARSENIC (PPM) [1]					
	Monday And/Or Preexposure Samples			Friday Samples		
	Mean	Range	% Observations > 0.3 ppm	Mean	Range	% Observations > 0.3 ppm
Colville National Forest	0.05	ND[2]-0.37	8	0.15	0.01-0.46	11
Okanogan National Forest	0.07	ND-0.50	9	0.24	0.02-1.30	33
Kaniksu[3] National Forest	0.01	ND-0.02	0	0.80	0.11-1.80	75
Wenatchee National Forest	0.03	ND-0.14	0	0.07	0.01-0.35	7

[1] Concentration of arsenic in urine from unexposed workers averaged 0.02 ppm for all groups.
[2] ND is not detectable (less than 0.01 ppm)
[3] Cacodylic acid

TABLE 2 A. VARIOUS BLOOD, KIDNEY AND OTHER CLINICAL MEASURMENTS IN EXPOSED AND CONTROL FOREST WORKERS, OKANOGAN NATIONAL FOREST, 1970

Clinical Test and (Units of Measurement)	Average Value [1]				
	Controls		Exposed		Normal Range
	June	August	June	August	
Arsenic in urine(ppm)	0.01[a]	0[b]	0.02[c]	0.04[abc]	———
SGOT[2] (units)	24	29	26	30	12-36
SGPT[3] (units)	18	24	21	20	6-53
LDH[4] (units)	359[a]	406	342[b]	430[ab]	200-500
Alk. Phos.[5] (units)	2.2	2.1	2.6	2.6	0.8-2.3
Creatinine (mg%)	1.2	1.2	1.2	1.2	0.9-1.4
BUN[6] (mg%)	16	14.9[a]	20[ab]	15[b]	8-22
Hematocrit (%)	48	49	47	49	40-54
Hemoglobin (g/100ml)	16	16	16	16	14-18
WBC[7] (10^3cells/ml)	7.1	8.0	7.0	7.9	5-10
PNC[8] (%)	52	56	54	58	50-70
Lymphocytes (%)	39	36	37	34	20-40
Monocytes (%)	4.1	4.9	5.6	5.1	1-6
Edsinophils (%)	3.4	2.2	0.4	3.1	1-5
Basophils (%)	0.8[ab]	0.2[b]	0[ac]	0.4[c]	0-1

Averages for a given test which have a lower case letter in common are significantly different at $p<0.05$

1/ Mean for nine men
2/ Transaminase
3/ Glutaminic Pyruvic
4/ Lactic Dehydrogenase
5/ Alkaline Phosphatase
6/ White Blood Count
7/ Blood Urea Nitrogen
8/ Polymorphonucleated Cells

TABLE 2B. VARIOUS BLOOD, KIDNEY AND OTHER CLINICAL MEASUREMENTS IN EXPOSED AND CONTROL FOREST WORKERS, COLVILLE NATIONAL FOREST, 1970

Clinical Test and (Units of Measurement)	Average Value [1]				
	Controls		Exposed		Normal Range
	June	December	June	December	
Arsenic in Urine(ppm)	0.01	0.02	0.01[a]	0.03[a]	——
SGOT[2] (units)	22[a]	29	22[b]	51[ab]	12-36
SGPT[3] (units)	14[a]	19[b]	18[c]	53[abc]	6-53
LDH[4] (units)	406[a]	480[b]	361[bc]	535[ac]	200-500
Alk. Phos.[5] (units)	1.9	1.6	1.8	2.1	0.8-2.3
Creatinine (mg%)	1.2	1.2	1.2	1.2	0.9-1.4
BUN[6] (mg%)	16	19	16	18	8-22
Hematocrit (%)	49	47	48	48	40-54
Hemoglobin (g/100ml)	16	16	16	16	14-18
WBC[7] (10^3cells/ml)	6.7[ab]	6.4[acd]	8.5[d]	8.4[bc]	5-10
PNC[8] (%)	58	55[a]	57	59[a]	50-70
Lymphocytes (%)	32	36	33	30	20-40
Monocytes (%)	5.8	4.7	5.3	6.5	1-6
Edsinophils (%)	3.9	3.0	3.2	3.1	1-5
Basophils (%)	0	0.5	0[a]	0.4[a]	0-1

Averages for a given test which have a lower case letter in common are significantly different at $p<0.05$

1/ Mean for eight men
2/ Transaminase
3/ Glutaminic Pyruvic
4/ Lactic Dehydrogenase
5/ Alkaline Phosphatase
6/ White Blood Count
7/ Blood Urea Nitrogen
8/ Polymorphonucleated Cells

TABLE 2 C: VARIOUS BLOOD, KIDNEY AND OTHER CLINICAL MEASUREMENTS IN EXPOSED AND CONTROL FOREST WORKERS

Kaniksu National Forest, 1970

Clinical Test and (Units of Measurement)	Average Value[1] Controls July	Controls August	Exposed July	Exposed August	Normal Range
Arsenic in urine(ppm)	0[a]	0.01[b]	0.01[c]	0.20[abc]	----
SGOT[2] (units)	27	23[a]	29[ab]	22[b]	12-36
SGPT[3] (units)	29[ab]	15[a]	19	14[b]	6-53
LDH[4] (units)	373	401	405	354	200-500
Alk. Phos.[5] (units)	2.2	2.1	2.6	2.4	0.8-2.3
Creatinine (mg %)	1.2	1.2	1.2	1.3	8-22
BUN[6] (mg %)	13	15	15	17	0.9-1.4
Hematocrit (%)	51[a]	49	50	49[a]	40-54
Hemoglobin (g/100ml)	17	17	16	16	14-18
WBC[7] (10^3cells/ml)	8.5	8.7	5.9	6.7	5-10
PNC[8] (%)	58[a]	59[bc]	47[ab]	50[c]	50-70
Lymphocytes (%)	33[a]	31[b]	44[ab]	39	20-40
Monocytes (%)	7	6.8	6.4	7.0	1-6
Edsinophils (%)	0.8[a]	1.4[b]	2.0	3.0[ab]	1-5
Basophils (%)	0.8	0.2	0.4	0.6	0-1

Averages for a given test which have a lower case letter in common are significantly different at $p<0.05$.

1/ Mean for five men
2/ Trensaminase
3/ Glutaminie Pyruvie
4/ Lactic Dehydrogenase
5/ Alkaline Phosphatase
6/ White blood count
7/ Blood urea nitrogen
8/ Polymorphonueleated cells

The data in Table 2 a, b, and c were subjected to analysis of variance and comparisons among mean values for specific clinical tests made by single degrees of freedom. All possible combinations of exposure and time of year were compared. Average values for a given test which have a lower case letter in common are significantly different at $p<0.05$. In Table 2a, which contains data for the Okanogan National Forest crew, there are several instances where clinical values are significantly different either between sampling times for the control or the exposed group, or between sampling times among exposure groups. In no case, however, do any values for the exposed group, fall outside the normal range. On the Colville National Forest (Table 2b), transaminase and lactic dehydrogenase enzyme activity in samples from exposed workers were significantly higher than controls, or preexposure levels in the exposed group. These values also fall outside the normal range. The relationship of these results to arsenic exposure seems remote because this pattern is not observed at study areas on the Okanogan or Kaniksu National Forests. It is particularly significant that similar differences were not found in the Kaniksu group since their average urine arsenic level at the end of the season were five and seven times higher than urine arsenic levels for Okanogan and Colville crews respectively. The Kaniksu crew did not show any statistically significant changes in clinical data for pre- and post-exposure measurements which fell outside the normal range. It is concluded there was no marked change in clinical data as a result of continued exposure to the silvicide.

Several crews from the Washington State Department of Natural Resources applied MSMA by the hack-squirt method in western Washington during the summer of 1970. Their urine arsenic levels were monitored regularly (Table 3). The limited data available show few observations exceed the 0.3 ppm arsenic level. The DNR crews used rubber gloves as opposed to the cotton work gloves used by the Forest Service crews in the 1969 and 1970 studies. It appeared from this study that the use of impervious gloves to prevent hand exposure could significantly reduce applicator urine arsenic levels.

TABLE 3: ARSENIC IN URINE FROM YOUNG FOREST WORKERS USING MSMA

Washington Department of Natural Resources, 1970.

	Arsenic (ppm)[1]					
	Monday and/or preexposure samples			Friday Samples		
	Mean	Range	% Observations 0.3 ppm	Mean	Range	% Observations 0.3 ppm
Cedar Creek Youth Camp	0.04	0.01-0.09	0	0.02	ND[2]-0.03	0
Naselle Youth Camp	0.04	ND-0.20	0	0.14	ND-0.54	14

1/ No data for unexposed workers.
2/ ND is not detectable (less than 0.01 ppm)

Arsenic In Blood and Urine Of Forest Workers Applying Dimethylarsinic Acid

Sheldon Wagner and Paul Weswig
Environmental Health Sciences Center
Oregon State University
Corvallis, Oregon

Wagner and Weswig measured arsenic in urine and blood from a Forest Service crew applying cacodylic acid on the Siuslaw National Forest during the summer of 1971. In contrast with Allard's study, where single urine samples were collected, Wagner and Weswig collected the total amount of urine voided during a 24-hour period and subjected this material to arsenic analysis. The results of this study have been prepared for publication and are in Appendix 3.

The Siuslaw Forest crew used in this study was made up of university students with a permanent Forest Service employee as foreman. The foreman received intensive indoctrination on the best procedures for handling and applying the silvicide in order to minimize the exposure of crew members and animals. The crew was carefully trained, and reviewed regularly the appropriate safe handling and application procedures. The use of face shields, skin cream, impervious gloves, nonspill equipment, and personal washing procedures minimized applicator exposure. The crew was highly motivated and the operation was carefully conducted throughout the thinning season. Strong, effective leadership was an important factor in the successful operation of this crew.

Wagner and Weswig did not find arsenic in urine in excess of 0.3 ppm in any 24-hour urine sample which indicates these workers were successful in minimizing their personal exposure. Blood-arsenic levels were not a good indicator of exposure to the silvicide.

The investigators feel 24-hour urine collections are preferred over random samples where urine volumes and salt concentrations may vary considerably. Urine volumes and salt concentrations are influenced to a large degree by the individual, the temperature and humidity, and the level of exertion. In earlier studies, these problems were adjusted for, in part, by collecting samples at approximately the same time each day and by measuring the osmolality of the urine and adjusting arsenic concentration proportionally to a predetermined osmolality. Twenty-four-hour urine collections are difficult in a field situation, and guidelines for safe levels of organic arsenic compounds in such samples are not readily available.

Relationship Of Arsenic Levels In Single and 24-Hour Urine Collections

Logan A. Norris
Pacific Northwest Forest and Range Experiment Station
Corvallis, Oregon

The purpose of this study was to find a relationship between arsenic levels in single and 24-hour urine samples. The objective was to relate Wagner and Weswig's 1971 study to the earlier studies and to devise a system of urine monitoring which is based on single urine samples rather than the cumbersome 24-hour urine sample collection method.

On a designated collection day, each crew member received one large and one small container. With the exception of one sample of urine taken at the end of the work day in the small container, all urine excreted during the 24-hour period was collected in the large container. At the end of the 24-hour collection period, both the large and the small containers were sent to the State of Washington Department of Social and Health services laboratory (Wenatchee laboratory) Wenatchee, Washington, for analysis.

The sum of the volumes of urine in each container equalled the total volume of urine voided during the 24-hour period. The arsenic concentration in the small sample was adjusted proportionally on the basis of the osmolality of large samples. The sum of the arsenic in the small and large samples was the total arsenic excreted during the 24-hour period. Creatinine was also measured as a guide to the volume and concentration of urine in the single and 24-hour samples. Thinning crews on the Colville National Forest participated in this study from 8/8/72 to 3/15/73 (Table 4).

The data for exposed forest workers are in Table 4. The data were subjected to multiple regression analysis. Micrograms (µg) arsenic excreted in 24 hours was the dependent variable and the following values for single urine samples were tried as possible independent variables: volume, osmolality, mg% creatinine and ppm arsenic.

The following regression equation was developed:

$$Y = 12.88 + 0.14\ (X_1) - 0.02(X_2) + 0.01(X_3) + 1386.1(X_4)$$

where

Y is µg arsenic excreted in 24 hours,
X_1 is ml urine in single samples,
X_2 is osmolality (mOs/kg) of single samples,
X_3 is mg % creatinine in single samples, and
X_4 is ppm arsenic in single sample.

$$R^2 = 0.76$$

The independent variables in this equation account for 76% of the variation in the dependent variable. Stepwise addition of independent variables in order of the importance of their contribution to the equation revealed that:

1. X_4, the concentration of arsenic in the single sample, accounted for 73.4% of the variation of Y and
2. X_4 and X_1, the concentration of arsenic and volume of urine in the single sample together accounted for 75.9% of the variation of Y.

Neither X_2, osmolality, nor X_3, creatinine mg %, in the single sample

TABLE 4: ARSENIC IN SINGLE AND 24 HOUR URINE SAMPLES FROM COLVILLE NATIONAL FOREST WORKERS APPLYING MSMA.

Worker No.	Sampling Date	Type of Sample	Urine Volume (ml)	Osmolality mOsm/kg	Creatinine mg%	Creatinine g/24 hr	Arsenic ppm	Arsenic µg/24 hr
110	8/8/72	single	598	1038	177	--	0.26	
		24 hr	1666	990	185	2.0	0.31	487
	8/22/72	single	490	960	135	--	0.16	
		24 hr	1772	874	120	1.6	0.15	271
	9/6/72	single	475	970	114	--	0.13	
		24 hr	1847	724	78	1.1	0.12	232
	9/19/72	single	422	1096	153	--	0.24	
		24 hr	1824	913	114	1.6	0.24	438
	10/18/72	single	117	1112	192	--	0.23	
		24 hr	1569	831	123	1.8	0.07	129
	11/8/72	single	472	1064	126	--	0.14	
		24 hr	1814	1048	141	1.9	0.11	214
	11/21/72	single	352	1184	174	--	0.28	
		24 hr	1434	1040	144	1.6	0.16	272
	12/6/72	single	360	966	231	--	0.22	
		24 hr	1580	955	153	1.9	0.20	323
	3/15/73	single	465	963	168	--	0.04	
		24 hr	1530	960	165	1.8	0.16	189

TABLE 4. (CONT)

Worker No.	Sampling Date	Type of Sample	Urine Volume (ml)	Osmolality mOsm/kg	Creatinine mg%	Creatinine g/24 hr	Arsenic ppm	Arsenic µg/24 hr
111	12/20/72	single	500	987	177	--	0.02	
		24 hr	2140	821	156	2.6	0.02	33
	3/14/73	single	410	879	159	--	0.03	
	3/15/73	24 hr	1475	950	201	2.1	0.03	44
112	8/8/72	single	425	958	195	--	0.04	
		24 hr	1287	1054	230	2.0	0.06	69
	8/22/72	single	312	853	135	--	0.02	
		24 hr	1894	811	132	2.1	0.03	54
	9/6/72	single	487	808	143	--	0.03	
		24 hr	1787	765	117	1.5	0.04	67
	3/15/73	single	285	876	156	--	0.02	
		24 hr	1415	812	144	1.6	0.04	51
113	8/8/72	single	262	1104	228	--	0.08	
		24 hr	832	1138	342	2.0	0.10	78
	8/22/72	single	380	1054	141	--	0.11	
		24 hr	1381	988	153	1.5	0.11	152

TABLE 4. (CONT.)

Worker No.	Sampling Date	Type of Sample	Urine Volume (ml)	Osmolality mOsm/kg	Creatinine		Arsenic	
					mg%	g/24 hr	ppm	µg/24 hr
115	11/8/72	single	352	1104	186	--	0.03	
		24 hr	1002	1132	183	1.2	0.03	30
	11/21/72	single	527	863	174	--	0.03	
		24 hr	1909	704	123	1.7	0.01	30
	12/6/72	single	540	497	87	--	0.01	
		24 hr	3160	794	147	3.9	0.02	58
116	8/22/72	single	532	402	87	--	0.01	
		24 hr	3232	446	96	2.6	0.01	32
	9/6/72	single	190	929	213	--	0.02	
		24 hr	982	558	102	0.81	0.02	20
	9/19/72	single	372	704	143	--	0.02	
		24 hr	2234	442	92	1.7	0.02	45
	10/18/72	single	212	996	231	--	0.04	
		24 hr	4034	179	36	1.4	0.01	47
	11/8/72	single	342	853	153	--	0.02	
		24 hr	1434	715	120	1.3	0.01	18
	11/21/72	single	312	951	177	--	0.05	
		24 hr	2254	454	93	1.8	0.01	35
	12/6/72	single	295	898	288	--	0.10	
		24 hr	2940	304	75	2.0	0.02	82
	3/15/73	single	225	892	231	--	0.03	
		24 hr	2625	767	201	4.8	0.04	103

TABLE 4. (CONT.)

Worker No.	Sampling Date	Type of Sample	Urine Volume (ml)	Osmolality mOsm/kg	Creatinine		Arsenic	
					mg%	g/24 hr	ppm	µg/24 hr
117	8/8/72	single	552	758	128	--	0.07	
		24 hr	1194	9.6	160	1.0	0.17	148
118	8/8/72	single	373	1090	156	--	0.11	
		24 hr	1715	802	108	1.4	0.13	215
119	8/8/72	single	482	924	63	--	0.04	
		24 hr	3284	813	38	1.1	0.05	159
	8/22/72	single	437	883	86	--	0.02	
		24 hr	3137	757	39	1.1	0.02	56
	10/18/72	single	452	982	111	--	0.02	
		24 hr	1524	954	102	1.1	0.02	30
	11/8/72	single	212	890	48	--	0.02	
		24 hr	2004	950	57	1.0	0.02	40
	11/21/72	single	432	935	84	--	0.06	
		24 hr	3354	740	24	0.70	0.02	84
	12/6/72	single	445	962	96	--	0.09	
		24 hr	1165	1006	117	0.84	0.06	83
120	12/20/72	single	308	662	210	--	0.05	
		24 hr	2370	366	69	1.4	0.01	36
121	3/15/73	single	360	876	165	--	0.03	
		24 hr	1300	839	150	1.4	0.12	124

TABLE 4. (CONT.)

Worker No.	Sampling Date	Type of Sample	Urine Volume (ml)	Osmolality mOsm/kg	Creatinine mg%	Creatinine g/24 hr	Arsenic ppm	Arsenic µg/24 hr
122	9/19/72	single	190	815	168	--	0.03	
		24 hr	2072	515	53	1.8	0.04	81
	10/18/72	single	482	786	150	--	0.02	
		24 hr	1905	544	96	1.4	0.03	52
	11/8/72	single	252	962	138	--	0.05	
		24 hr	1174	948	144	1.3	0.07	77
	11/21/72	single	162	895	171	--	0.08	
		24 hr	1294	754	117	1.3	0.06	81
	12/6/72	single	450	915	189	--	0.06	
		24 hr	1560	832	150	1.7	0.04	71
123	12/20/72	single	478	458	72	--	0.03	
		24 hr	2198	424	99	1.7	0.02	49
	3/15/73	single	378	461	105	--	0.04	
		24 hr	2178	382	75	1.4	0.06	123
124	8/22/72	single	272	999	186	--	0.04	
		24 hr	1394	944	164	1.8	0.06	78
	9/6/72	single	270	818	141	--	0.19	
		24 hr	1802	795	81	1.2	0.23	404
	9/19/72	single	380	925	194	--	0.14	
		24 hr	2452	536	91	1.9	0.13	323

TABLE 4. (CONT.)

Worker No.	Sampling Date	Type of Sample	Urine Volume (ml)	Osmolality mOsm/kg	Creatinine		Arsenic	
					mg%	g/24 hr	ppm	µg/24 hr
124	10/18/72	single	322	899	132	--	0.06	
		24 hr	1814	886	129	1.9	0.09	154
	11/8/73	single	252	1082	105	--	0.15	
		24 hr	1284	1066	141	1.5	0.15	193
	11/21/73	single	452	652	90	--	0.08	
		24 hr	1334	914	180	1.6	0.11	133
	12/6/73	single	500	877	168	--	0.05	
		24 hr	1550	868	174	1.8	0.07	98
125	8/8/72	single	237	1076	360	--	0.14	
		24 hr	1859	521	158	2.6	0.12	228
126	8/8/72	single	292	1122	205	--	0.08	
		24 hr	1116	1180	170	1.4	0.24	221
127	12/20/72	single	490	293	57	--	0.03	
		24 hr	2220	341	72	1.3	0.03	67
	3/15/73	single	272	326	63	--	0.01	
		24 hr	2112	288	57	1.1	0.03	58

contributed significantly to the regression equation.

The results of this study indicate single urine samples taken at the end of the work day can be used in place of 24-hour urine collections in operational monitoring programs. The volume and concentration of arsenic in the single sample are useful predictors of this amount of arsenic excreted in 24 hours according to the following equation:

micrograms (μg) arsenic/24 hours = -12.88+1386.1 (ppm arsenic, single sample)+0.14 (ml urine, single sample)

Pattern Of Arsenic Elimination In Urine Of Applicators During One Work Week

Logan A. Norris
Pacific Northwest Forest and Range Experiment Station
Corvallis, Oregon

During the summer of 1971, tests were conducted to determine the pattern of arsenic elimination during the work week. All earlier studies involved samples collected on Monday mornings or Friday evenings which showed Friday levels were higher than Monday levels but that levels dropped quickly from Friday to the next Monday. This study asked the following questions:

1. How quickly does the concentration of arsenic in urine rise during the work week?
2. Does the concentration drop between the evening sample on one day and the morning sample on the following day?
3. When is the earliest time in the work week when samples of urine should be collected for arsenic determinations?

Intensive monitoring of urine was conducted over a 10-day period using two thinning crews on the Colville National Forest and one crew on the Siuslaw National Forest. Individual samples of urine were collected from each individual at the end of the work day on Friday and in the morning and afternoon of each day during the next week. A final set of individual urine samples was collected before the start of the work day on the next Monday. Samples of urine were shipped to the Wenatchee laboratory where composite samples for a given crew for a given collection time were prepared. The concentration of arsenic and the osmolality on composite samples were determined and the concentration of arsenic adjusted proportionally to a common osmolality of 737 mOs/kg which was the mean value for all observations (Table 5).

The success of this study depended on applicators exposing themselves to the silvicide. The Colville crews did not. Their use of protective gear and careful chemical handling and application procedures are reflected by the low urine arsenic levels observed. The mean arsenic concentrations were 0.03 ppm and 0.02 ppm for a.m. and p.m., respectively, for the exposed crew. These differences are too small to permit the comparisons desired. The Siuslaw crew exhibited slightly higher urine arsenic levels, but no definitive pattern of arsenic excretion is apparent except that concentrations seem higher later in the week. Data from only one crew does not provide a sufficient base for suggesting an alternate urine sampling time.

Table 5.--Arsenic in urine of applicators at various times during the work week. Colville and Siuslaw National Forests, 1971.[1/]

		Arsenic in urine (ppm)[2/]					
		Colville Crew I		Colville Crew II		Siuslaw Crew	
Day	Time	Control	Exposed	Control	Exposed	Control	Exposed
Friday	pm	0.02	0.08	0.03	0.03	0.02	0.01
Monday	am	0.03	0.03	0.03	0.02	0.05	0.02
	pm	0.02	0.06	0.03	0.03	0.02	0.05
Tuesday	am	0.01	0.09	0.03	0.04	0.05	0.05
	pm	0.01	0.09	0.01	0.03	0.04	0.10
Wednesday	am	0.02	0.05	0.02	0.04	0.03	0.07
	pm	0.02	0.06	0.02	0.05	0.02	0.01
Thursday	am	0.03	0.07	0.07	0.03	0.03	0.26
	pm	0.02	0.04	0.01	0.04	0.02	0.10
Friday	am	0.06	0.07	0.03	0.04	0.05	0.12
	pm	0.04	0.04	0.02	0.03	0.05	0.28
Monday	am	0.02	0.01	0.01	0.04	0.05	0.02

1/ Arsenic in composite urine samples from thinning crew. Value represents in essence a crew average value.

2/ Corrected for difference in osmolality on a proportional basis to a base of 737 mOsm/kg, the mean for all observations.

How Much Arsenic is Permissible in Urine From Applicators?

Considerable emphasis is placed in this report on the level 0.3 ppm arsenic in urine. The following statement is from an undated bulletin from the Chemical Products Division of the Ansul Co., Marionette, Wis.

" The Saftey and Handling of Ansul Herbicides"

> " Because some individuals are more sensitive than others it is advisable to always be on the lookout for signs of skin sensitivity among persons handling arsenicals. When a person shows sensitivity, he should be removed from exposure. When an individual is exposed daily for extended periods, the inspection for skin sensitivity should be supplemented by monthly urine analysis for arsenic. Some state health departments or commercial laboratories are usually equipped to do this analysis. When a sample shows above about 0.3 milligrams of arsenic per liter[1/], another sample should be taken, and if again above this figure, the donor should be removed from exposure. Actual experience with Ansul personnel and our manufacturing operation shows that removal from exposure quickly reduces the urine arsenic level to normal and eliminated the signs of skin sensitivity."

The adequacy of 0.3 ppm arsenic in urine as an indicator of exposure to a potentially dangerous dose of organic arsenicals is uncertain. A letter from Dr. P. J. Ehman, Ansul Company, considers this question (Appendix 4). The 0.3 ppm arsenic in urine level may be too restrictive since evidence of arsenic intoxication accompanying urine arsenic levels slightly greater than 0.3 ppm is lacking. Wagner, Weswig, and Allard have all suggested arsenic in urine greater than 0.3 ppm could probably be tolerated, but dose response data is lacking. Futhermore, current thinning practices and procedures have resulted in urine arsenic levels which only infrequently exceed 0.3 ppm. We have learned to live with the 0.3 ppm level. In view of the uncertainty about an alternate value and the inadvisability of seeming to encourage applicator exposure by raising the permissable level, the 0.3 ppm arsenic in urine level should be retained.

1/ 0.3 ppm

SUMMARY OF STUDIES OF EXPOSURE AND EFFECTS ON HUMAN HEALTH

Tarrant and Allard found that forest workers applying MSMA and cacodylic acid were receiving considerable exposure to the arsenicals. Arsenic in urine proved to be a valuable index to exposure level. In 1970, Allard measured arsenic in urine and collected clinical data on body biochemistry for applicators. These crews had received intensive orientation to the use of the chemical. Generally low urine arsenic levels attested to the importance of proper crew orientation. Few significant deviations in clinical data were noted for the study crews. DNR crews operating during this same period used rubber gloves instead of cotton gloves and had low urine arsenic levels. Wagner and Weswig used a 24-hour urine collection period in 1971 instead of the single sample collection technique used previously. He measured arsenic in urine and blood of cacodylic acid applicators on the Siuslaw National Forest. This well-trained, highly motivated and effectively supervised crew had exceptionally low urine arsenic levels. Blood was not a good indicator of arsenic exposure.

No discernible pattern of arsenic excretion was observed during intensive urine monitoring during a work week. Applicators were so successful in minimizing their personal exposure that the objectives of the study were not attained. In another study, Norris established that urine volume and arsenic concentration in single urine samples could be used to predict total arsenic excreted in 24 hours. Thus single samples may be used in operational monitoring.

A sound basis for establishing a permissible urine arsenic value greater than 0.3ppm has not been found. In view of the success operational crews have had in meeting this value, no change is suggested.

Differences in the arsenic levels in samples taken in 1969 and 1970 from Forest Service crews, and in the 1970 samples from the DNR crew, suggested that intensive training in the handling and application of the silvicide and the use of adequate protective gear and close supervision of the application can markedly reduce arsenic exposure to the applicator.

BEHAVIOR OF ARSENICALS IN THE FOREST

Three studies were conducted which considered the movement, persistence, and fate of the arsenicals in the forest. These studies establish the nature of the exposure likely to be received by animals in thinned areas and the entry of arsenic to ground and surface waters.

Leaching of Cacodylic Acid and MSMA in Forest Floor Material and Soil

Logan A. Norris
Pacific Northwest Forest and Range Experiment Station
Corvallis, Oregon

The purpose of this study was to determine the leaching characteristics of the organic arsenicals in forest floor material and soil, and the likelihood the use of these chemicals well result in water contamination. The following forest floor materials and soils were used.

Forest floor material (l, F, and H horizons)	Source
1. Pondersoa pine	Bend, Oregon
2. Douglas-fir	Vernonia, Oregon
3. Mixed fir	Colville, Washington

Soil 0-6" horizon	Source
1. Klicker, Basaltic silt loam	LaGrande, Oregon
2. Astoria, Sedimentary silt clay-loam	Vernonia, Oregon
3. Edds, Loam over glacial till	Colville, Washington

Additional information on the chemical and biological properties of these materials are in Table 27.

To determine the leaching characteristics of MSMA and cacodylic acid, glass columns, 13 cm in diameter, were packed to a depth of 7.62cm (3 in.) with each of the study materials. The columns were leached with water overnight, and then 500 micrograms (µg) of arsenic as cacodylic acid or MSMA applied to the top of the columns. Water was then added to the columns in 0.5 ml increments, with 45 minutes between applications. A total of 6 ml was added each day (equivalent of 4.3 cm (1.7 in.) of water per day). Water was collected as it dripped from the bottom of the columns and was analyzed for arsenic. Each test column was leached with a total of 86.4 cm (34 in.) of water. Results of the leaching experiment are in Table 6.

Both MSMA and cacodylic acid were rapidly leached through forest floor material. Chemical deposited on the surface of the forest floor

TABLE 6: ARSENIC FROM CACODYLIC ACID OR MSMA LEACHED THROUGH 7.62 cm (3 in.) OF FOREST FLOOR MATERIAL OR SOIL. COLUMNS RECEIVED 4.3 cm (1.7 in.) WATER PER DAY FOR 30 DAYS.

Cumulative arsenic recovery in leachate (% of arsenic applied to column).

Leachate (Cumulative Inches)	Forest Floor						Soil					
	Douglas-fir		Ponderosa pine		Mixed fir		Astoria		Klicker		Edds	
	Caco.	MSMA	Caco.	MSMA	Caco.	MSMA	Caco.	MSMA	Caco.	MSMA	Caco.	MSMA
2.8	22	40	53	40	40	15	0	0	2	0	1	1
5.7	70	72	72	73	70	63	0	0	6	1	2	2
8.5	82	82	80	80	79	75	1	1	12	1	3	2
11.3	86	86	83	83	83	78	1	1	20	1	5	2
14.1	88	87	84	85	85	79	1	2	30	2	8	3
17.0	89	87	84	85	86	80	2	2	40	2	13	3
19.8	90	88	84	85	88	81	2	3	50	2	18	4
22.6	90	89	84	86	88	81	2	3	60	3	23	5
25.5	91	90	85	86	89	81	3	4	68	3	30	6
28.3	91	90	85	88	90	82	3	4	72	4	36	6
31.1	92	91	86	88	91	83	3	5	80	4	43	7
34.0	92	92	87	89	91	84	4	6	84	5	48	8

should readily move to the mineral soil with even small amounts of precipitation.

MSMA was virtually immobile in the three soils tested. Cacodylic acid was quite immobile in the Astoria soil, but showed some movement in both the Edds and Klicker soils. In Klicker soil, 86.4 cm of water were required to move 84% of the cacodylic acid 7.62 cm, while the same amount of water moved about 50% of the chemical 7.62 cm in Edds soil. These are short distances relative to the distances from treated areas to groundwater or streams. The conclusion is neither MSMA nor cacodylic acid are sufficiently mobile in soil to cause significant contamination of groundwater.

In another set of tests, undisturbed 2.54 cm (1 in.) deep cores of forest floor were placed in columns and 1 mg of arsenic as MSMA or cacodylic acid added to the surface. Water was applied as a fine mist to the surface of the cores three times a day to equal .32 cm (1/8 in.) of water per day. The water which drained through the bottom of the cores was collected and analyzed for arsenic. The results are expressed as the cumulative percentage of the material which was recovered in 0.33 cm increments of water (Table 7).

The results confirm both MSMA and cacodylic acid are highly mobile in forest floor material. As little as one inch of rain delivered over an eight-day period was sufficient to move approximately half of these materials through one inch of forest floor material. MSMA and cacodylic acid which are accidently deposited on the forest floor will be readily moved through the forest floor to the soil by even small amounts of precipitation. MSMA is nearly completely immobile in soil. Cacodylic acid is slightly more mobile in soil than MSMA, but its movement is also very limited. Neither MSMA nor cacodylic acid pose significant hazard to groundwater quality in areas treated with these materials for pre-commercial thinning.

TABLE 7: ARSENIC LEACHED THROUGH ONE-INCH CORES OF UNDISTURBED FOREST FLOOR BY 0.33 CM. DAILY INCREMENTS OF WATER.

Leachate

Cumulative Inches	Douglas-fir		Ponderosa pine		Mixed fir	
	Caco.	MSMA	Caco.	MSMA	Caco.	MSMA
0.13	19	14	20	20	30	22
0.26	36	23	38	37	42	27
0.39	43	25	44	42	46	32
0.52	49	30	48	45	52	36
0.65	54	35	53	48	55	42
0.78	58	38	56	51	58	44
0.91	62	42	59	55	62	46

DISTRIBUTION OF ARSENIC IN VEGETATION, FOREST FLOOR, SOIL, AND STREAMWATER AFTER THE INJECTION OF FOREST TREES WITH MSMA AND CACODYLIC ACID

Paul R. Canutt
U.S.F.S., Region 6, Portland, Oregon

Logan A. Norris
Pacific Northwest Forest and Range Experiment Station
Corvallis, Oregon

The purposes of this study were to determine (1) the concentration of arsenic in several parts of the forest environment after treatment of individual trees and (2) the concentration of arsenic in streams flowing through areas thinned in operational scale treatments. A total of three study areas were established in larch, ponderosa pine, and Douglas-fir forest types on the Colville and Wenatchee National Forests.

At each study site, five trees were selected for injection with MSMA at normal rates. Trees were separated from each other by not less than eight times the crown radius of the nearest, largest treated tree. Each tree served as a plot center from which pretreatment and post-treatment data were collected. Sampling transects were projected at 0, 120, and 240 degrees from the bole of each treated tree. Three sampling points were established on each transect at 0.5 X crown radius 2 X crown radius, and 4 X crown radius from the bole. Samples collected at a given distance from the bole were composited for the three transects, the composite samples were analyzed for arsenic at the Wenatchee laboratory.

Four types of sample materials were collected at each study site: Forest floor, (L, F. and H horizons), mineral soil (0-12 inches), browse and herbage. Forest floor material was collected from an 8-inch square plot to mineral soil. The weight of the forest floor material was determined and arsenic levels expressed as mg arsenic per square foot and ppm. Herbage samples included only above ground portions of the plants. Browse was the terminal four inches of branches of browse species. The average arsenic levels for the five trees for each of the study areas are summarized in Tables 8, 9, and 10.

The study was designed as a random block split plot experiment with distance from the tree as whole plots and collection time and distance-time interactions as split plots. The data were subjected to analysis of variance and differences among specific treatments compared by Scheffe' test.

At the Ellensburg study site ponderosa pine type,(Wenatchee National Forest), there was no statistically significant change in arsenic concentration in herbage or browse as a function of time (Table 8). What appear to be small increases in arsenic concentration in the fall of 1970 may be the result of chance sampling. There was a statistically significant linear decrease in arsenic concentration in herbage with increasing distance from the tree but a similar pattern was not detected for browse. The concentration of arsenic in forest floor decreased with distance between 0.5 and 2.0 X crown radius from the tree. There was no difference in arsenic concentrations in forest floor in samples collected at two and four times the crown radius. The large change in arsenic concentration in forest floor with time was significant and followed a cubic function. Changes in arsenic concentration in soil

TABLE 8: ARSENIC RESIDUES IN ENVIRONMENTAL SAMPLES FROM ELLENSBURG STUDY AREA[1/]

Sample Type	Distance from treated stem	Arsenic concentration(ppm) [2/] (Pretreatment) Spring 1970	General Sample Time Fall 1970	Spring 1971	Spring 1972
Herbage	1/2 crown radius	0.26	1.34	o.74	0.60
	2 crown radius	0.14	2.00	0.36	0.52
	4 crown radius	0.18	0.18	0.28	0.22
Browse	1/2 crown radius	o.24	1.80	0.44	0.68
	2 crown radius	0.30	0.30	0.34	0.58
	4 crown radius	0.28	0.28	0.40	0.42
Forest Floor	1/2 crown radius	0.50 (0.28)[3/]	1.18 (0.49)	43.24 (18.33)	9.00 (1.53)
	2 crown radius	0.92 (0.98)	0.54 (0.32)	2.76 (1.82)	0.70 (0.15)
	4 crown radius	0.54 (0.38)	0.65 (0.41)	0.72 (0.44)	0.64 (0.18)
Soil, 0-12"	1/2 crown radius	1.70	1.60	2.00	2.34
	2 crown radius	1.90	1.56	2.30	1.92
	4 crown radius	1.56	1.58	2.32	1.96

1/ Ponderosa pine forest type, Wenatchee National Forest.

2/ Mean of five trees.

_/ Values in() are mg As per square foot.

TABLE 9: ARSENIC RESIDUES IN ENVIRONMENTAL SAMPLES FROM COLVILLE STUDY AREA[1/]

Sample type	Distance from treated stem	Arsenic Concentration (ppm)[2/] (Pretreatment) Spring 1970	Fall 1970	General Sample time Spring 1971	Spring 1972
Herbage	1/2 crown radius	0.28	0.32	0.52	0.66
	2 crown radius	0.26	0.20	0.40	0.30
	4 crown radius	0.22	0.26	0.42	0.68
Browse	1/2 crown radius	0.22	0.30	0.74	0.46
	2 crown radius	0.36	0.18	0.48	0.58
	4 crown radius	0.30	0.16	0.66	0.52
Forest floor	1/2 crown radius	0.72 (.62)[3/]	2.92 (2.97)	1.82 (2.08)	1.82 (3.50)
	2 crown radius	0.56 (.58)	2.68 (2.04)	1.78 (1.87)	1.86 (7.11)
	4 crown radius	0.62 (.52)	2.36 (2.34)	1.48 (1.43)	1.46 (4.51)
Soil, 0-12"	1/2 crown radius	3.34	3.54	3.08	3.62
	2 crown radius	3.20	4.58	3.84	3.34
	4 crown radius	4.02	4.20	3.62	3.16

1/ Larch forest type, Colville National Forest

2/ Mean of five trees.

_/ Values in () mg As per square foot.

TABLE 10: ARSENIC RESIDUES IN ENVIRONMENTAL SAMPLES FROM LAKE WENATCHEE STUDY AREA[1/]

Sample type	Distance from treated stem	Arsenic Concentration (ppm)[2/] (Pretreatment) Summer 1970	General Sample Time Fall 1970	Summer 1971	Summer 1972
Herbage	1/2 crown radius	0.18	1.72	0.64	0.50
	2 crown radius	0.18	0.26	0.30	0.40
	4 crown radius	0.10	0.26	0.28	0.34
Browse	1/2 crown radius	0.26	0.24	0.34	0.78
	2 crown radius	0.22	0.26	0.24	0.48
	4 crown radius	0.42	0.24	0.36	0.66
Forest floor	1/2 crown radius	0.76 (2.19)[3/]	3.08 (7.61)	3.42 (3.84)	1.52 (1.73)
	2 crown radius	0.64 (2.18)	1.02 (1.92)	1.30 (1.40)	1.38 (2.09)
	4 crown radius	0.82 (1.70)	1.02 (2.61)	0.84 (0.83)	0.60 (0.93)
Soil, 0-12"	1/2 crown radius	1.02	1.22	2.12	2.04
	2 crown radius	0.94	1.24	1.82	1.88
	4 crown radius	1.02	1.24	1.62	2.00

1/ Douglas-fir forest type, Wenatchee National Forest

2/ Mean of five trees.

3/ Values in () ar mg As per square foot.

were relatively small. The Ellensburg study area had been scarified some time before treatment and relatively little forest floor material was present before treatment. Consequently the fresh fall litter from treated trees were not "diluted" by old litter from trees prior to treatment and the increased concentration of arsenic in forest floor magnified. There was no significant differences with distance from the tree, but concentrations did follow a cubic function with time.

At the Colville study area, (Colville National Forest), the concentration of arsenic in both herbage and browse increased with time after treatment although the response in herbage was linear while the response in browse was cubic (Table 9). By spring 1972, the arsenic concentration in grass was still higher than in pretreatment samples but the same apparent difference in browse was not statistically significant. In forest floor, arsenic concentration followed a cubic function with time with peak concentrations occuring the fall after treatment. There was no change in forest floor arsenic concentration between 1971 and 1972 at this study site. The increase in arsenic concentration in forest floor is not reflected in the soil. There was no change in arsenic concentration in soil from Colville with either time after application or distance from the tree.

At the Lake Wenatchee study site, (Wenatchee National Forest) the arsenic concentration in herbage and browse increased with time according to cubic and quadratic functions, respectively (Table 10). Herbage concentration peaked in the fall of 1970 while browse concentrations did not show any statistically significant change until the summer of 1972. The concentration of arsenic in both herbage and browse decreased with distance from the tree according to quadratic functions. In the forest floor and soil, arsenic concentrations increased with time according to quadratic and linear functions respectively which suggests a continued input of arsenic from the crowns. The concentration of arsenic in forest floor decreased linearly with distance from the stem, but there was no similar pattern in the soil.

In general, there were only relatively small increases in arsenic concentrations in herbage and browse after treatment. The residues in vegetation in this study are relatively minor in comparison to dosages used in toxicological studies of Harr, Dickinson and Evans which are reported later. Harr found domestic rabbits survived with few problems on a diet containing 27 ppm arsenic as MSMA. The animals showed conplete recovery in 5 weeks when removed from the arsenic-containing diet. The arsenic residues found in this study are ten to one hundred times less than the levels employed by Harr.

Dickinson's cattle were injured by oral dosages approaching 100 mg/kg MSMA (45,000 mg MSMA for a 450 kilogram [990#]cow) in a 10-day period. If a cow consumes 10 percent of its body weight in feed per day during a 10-day grazing period, the feed must contain approximately 47 ppm arsenic to be equivalent to the dosage used by Dickinson. The arsenic residues found in this study are 20 to 500 times lower.

Evans reported an acute oral LD_{50} for snowshoe hare at 175 mg arsenic per kilogram body weight. Assuming 1/10 the LD_{50} is a no-effect level, and a rabbit consumes 10 percent of its body weight per day in feed, Evans' dose is the equivalent to feed containing 175 ppm arsenic. The residue in this study are 100 to 1,000 times less than this value.

The Food and Drug Administration has established tolerances the equivalent of 0.6 ppm and 2.0 ppm arsenic for MSMA and cacodylic acid, respectively in cotton seed hulls used for cattle feed.* Most of the arsenic residues found in vegetation in this study fall within these tolerances.

Changes in arsenic concentration in forest floor largely reflect the time of needle fall and distance from the tree. With the exception of the Ellensburg stand, increases in arsenic concentration and forest floor were far less than anticipated. Levels of arsenic in forest floor at the Ellensburg study area were magnified by lack of "pretreatment" forest floor because the site had been scarified. All study areas show a decline in arsenic concentration with time. The fate of arsenic in the forest floor is not known for centain, but small increases in soil residues indicate some movement from forest floor to the soil. Newton's work suggests vapor losses of arsenic may account for some changes in arsenic levels at the study sites.

Examination of the amounts of arsenic in forest floor expressed on a square foot basis suggests significant losses occured at the Ellensburg study site between 1971 and 1972. Only small changes, however, occurred at Lake Wenatchee and a moderate increase occurred at the Colville study site.

In the second part of this study, water samples from streams flowing through areas that were operationally thinned were tested for changes in arsenic concentration. Monitoring sites were located in the Colville, Wenatchee, and Kaniksu National Forests. Sample sites were located upstream (control) and downstream from the treatment units. Samples were collected at various intervals after treatment with emphasis given to storm periods when runoff events might occur and again during the spring runoff period. Water samples were analyzed at the Wenatchee Laboratory. Figures 1 and 2 and Tables 11 and 12 show the treatment areas, sampling locations, and results.

The results of the water sampling demonstrates clearly the lack of entry of significant arsenic residues to streams flowing through the study sites treated with MSMA or cacodylic acid. Only five samples contained detectable quantities of arsenic. Four of these five are right at the minimum detectable limits and the fifth sample represents an upstream site presumably containing water which has not passed through areas previously thinned with organic arsenicals. The results of this study indicate the careful application of MSMA and cacodylic acid to forest areas pose little or no hazard to aquatic organisms or downstream water users dependent on water flowing through chemically thinned areas.

* Federal register: December 3, 1970, and January 18, 1972.

FIGURE 1

COLVILLE N.F. TREATMENT AREAS

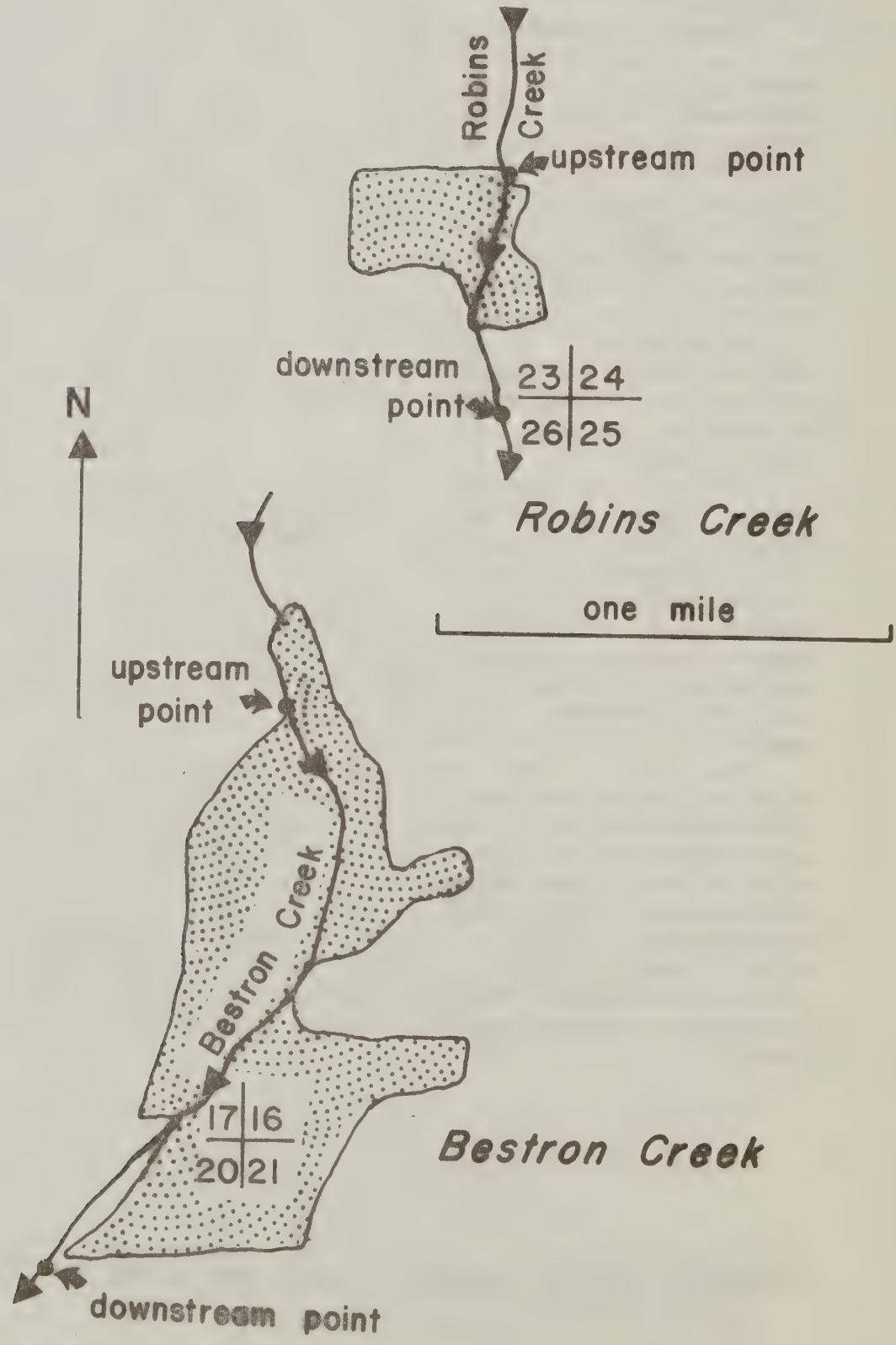
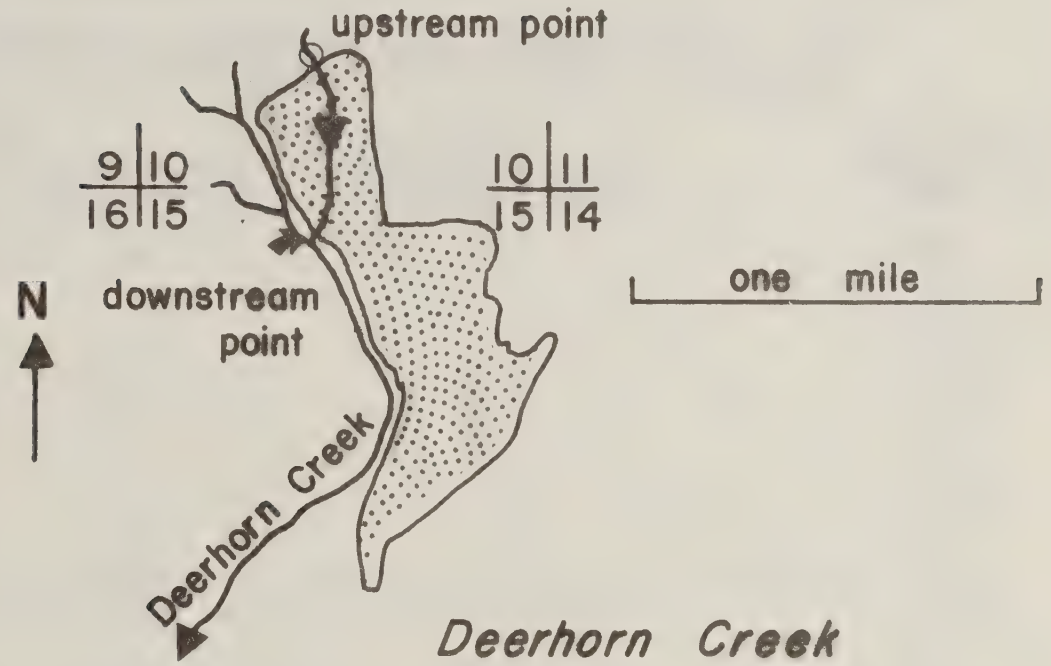


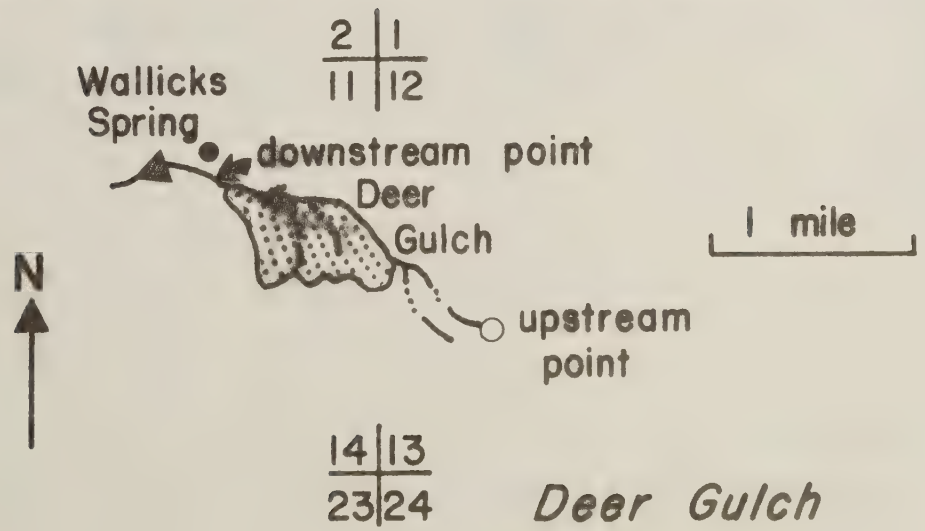
FIGURE 2

KANIKSU N.F. TREATMENT AREA



Deerhorn Creek

WENATCHEE N.F. TREATMENT AREA



Deer Gulch

Table 11.--Arsenic residues in streams flowing through chemically thinned areas, Colville National Forest.

Robbins Creek, treated with MSMA, October 12, 1970 to December 2, 1970.

Sampling Date	Upstream Site Arsenic (ppm)	Downstream Site Arsenic (ppm)
10/13/70	ND[1/]	ND
11/23/70		↓
12/14/70		
3/8/71		0.01
3/29/71		ND
4/3/71		
4/10/71		
4/11/71		
4/24/71		
5/4/71		
5/8/71		
5/27/71		
5/31/71	↓	↓

Bestrom Creek, treated with MSMA, December 3, 1970 to January 28, 1971.

Sampling Date	Upstream Site Arsenic (ppm)	Downstream Site Arsenic (ppm)
11/30/70	ND	ND
12/14/70	↓	↓
3/8/71	0.03	0.01
3/29/71	ND	ND
4/3/71		0.01
4/10/71		ND
4/17/71		
4/24/71		
5/4/71		
5/8/71	↓	↓

1/ ND means not detected (less than 0.01 ppm).

TABLE 12: ARSENIC RESIDUES IN STREAMS FLOWING THROUGH CHEMICALLY THINNED AREAS.

Tributary of Deerhorn Creek, Kaniksu National Forest. Treated With Cacodylic Acid, July 27, 1970 to August 21, 1970.

Sampling Date	Upstream Site Arsenic (ppm)	Downstream Site Arsenic (ppm)
7/22/70	ND[1/]	ND
8/13/70	↓	↓
8/26/70	↓	↓
9/4/70	↓	0.01
9/23/70	↓	ND
10/27/70	↓	↓
5/25/71	↓	↓

Deer Gulch, Wenatchee National Forest. Treated With MSMA, July 14, 1970 to September 3, 1970.

Sampling Date	Wallicks Spring Arsenic (ppm)	Upstream Site Arsenic (ppm)	Downstream Site Arsenic (ppm)
7/3/70	ND	ND	ND
8/5/70	↓	↓	↓
8/29/70	↓	↓	↓
9/13/70	↓	↓	↓
9/27/70	↓	↓	↓
10/15/70	↓	↓	↓
10/30/70	↓	↓	↓
5/3/71	↓	↓	↓

1/ ND means not detected (less than 0.01 ppm).

FATE OF ORGANIC ARSENIC HERBICIDES IN CHEMICALLY THINNED FORESTS OF WASHINGTON AND OREGON

Michael Newton and Robert Greaves
School of Forestry, Oregon State University
Corvallis, Oregon

The objectives of this study are to determine the contribution of treated stands to arsenic levels in the forest environment and to determine how long and in what form arsenic persists in the soil. The research is split about equally between field and laboratory studies. The following specific questions are asked:

1. What is the arsenic budget in trees injected with cacodylic acid or MSMA?
2. How much arsenic reaches the forest floor in freshfall litter in treated stands?
3. How long and in what form does arsenic persist in the soil?
4. What are the metabolic products of microbial attack on organic arsenicals?

The first studies conducted were in the laboratory to determine the metabolism of MSMA and cacodylic acid by molds. Czapek-dox agar containing various levels of arsenicals were innoculated with molds cultured from wood. Some of the agar was glucose-free while some was provided with glucose as an energy source. After two weeks, cultures were checked by smell for evolution of the garlicky odor characteristic of arsine or cacodyl. It was observed that those cultures supporting colonies of molds, and containing glucose, were producing the characteristic odor from substrate containing up to 50,000 parts per million MSMA. These finding stimulated another experiment to investigate the relation between the energy source in the substrate and the ability of molds to produce arsine from both cacodylic acid and MSMA in various concentrations.

A factorial design was used to investigate the loss of arsenic, through volatilization, as the function of arsenic concentration and energy availability. The experiment had four objectives: 1) to confirm quantitatively the loss of arsenic due to biological reduction of organic arsenicals, 2) to determine rates of degradation as the function of energy source, 3) to formulate a mathematical model of the kinetics of biological degradation based on bonding energy and biological efficiency, and to formulate a general model for arsenic reduction, based on molecular structure of both beginning and end points, considering sources of energy used for reduction. At this time, the first two objectives have been partially fulfilled.

Laboratory procedure involved preparing media with a range of arsenical and glucose concentrations, inoculation with a mixture of organisms, incubation for various periods, drying, and analysis by neutron activation. Media were prepared from Czapek-Dox agar so that each concentration of arsenical would be present in glucose concentrations of 0.3, 1.0, 3.0 and 10.0 percent by weight. Concentrations of methanearsonic acid and cacodylic acid were prepared so that each glucose concentration was spiked with no arsenical, or with no arsenical, or with concentrations of 10^2, 10^3, or 10^4 parts per million of arsenic as the organic compound. There were thus sixteen combinations of arsenical and glucose for each herbicide.

Each was replicated five times.

All dishes were inoculated with a suspension of dust from a bark-processing laboratory, then placed in the incubator with incubation temperature set at 25° Celsius. At various intervals, randomly selected samples were removed and placed in the freezer to prevent biological activity; a complete subset of samples from each herbicide was removed at each date.

After all samples had been removed from the incubator (31 days), all were removed from the freezer, photographed, dried at 55° C. and processed for neutron activation. Activation involved irradiation at either 100 or 1,000 kilowatts, depending on arsenic level. This provided adequate counting resolution with minimal sodium interference.

Findings

Arsenic remaining in each sample was determined on an absolute scale, and converted to percent of the original concentration. Table 13 illustrates mean arsenic percent remaining for both herbicides, average of all glucose levels, for various lengths of incubation time.

The rate of degradation did not appear to be the same for all concentrations either of glucose or arsenic. The rate of loss is portrayed in Table 14 as the function of concentration of both arsenic and glucose. Because the modeling is not yet complete, a refined statistical interpretation is not available at this time.

Despite the interaction of energy source and arsenic level, it was observed that substantial losses of arsenic occurred with all levels of glucose. The evidence was virtually irrefutable that these losses occurred through volatilization at temperatures below 70°C. The losses were clearly defined only at the highest levels of arsenic, however, with variation among lower levels obscuring major changes.

There is no clear explanation for variation in rate of reduction. Inoculation was remarkably uniform for all replications of both chemicals. There was a strong interaction between glucose and arsenical concentration that amounted to a substrate-organism specifically. It is possible that some of the communities of organisms were more able to reduce arsenicals than others. One of the molds, however, was uniformly present in all the MAA plates of 10^3 and 10^4 ppm, and in cacodylic acid plates of high glucose and 10^2 ppm or less arsenic.

High concentrations of cacodylic acid were colonized by bacteria and actinomycetes only, except for occasional isolated mold colonies. Bacteria were suppressed either by the MAA in high concentrations, or by the presence of molds. In any event, the degree of colonization undoubtedly influenced rate of reduction.

There is no obvious indication of contamination that would explain the recorded increases in arsenic concentration in the lower levels of cacodylic acid. It is possible that arsine concentrations in the atmosphere in which they were incubating could have caused some migration, but the same pattern should have been noted with MAA as in cacodylic acid if this were the case. Considering that a percentage unit at the 10^4 concentration is a full milligram of loss, and that a percentage unit at 10^2 concentration is 10 micrograms, the precision of estimate at the higher levels with neutron activation is ten times as great. Thus, it is anticipated that confidence can be placed in the loss estimates at high concentrations, but that greater precision is needed at the lower levels.

TABLE 13.--PERCENT REMAINING OF ARSENIC OF VARIOUS INITIAL CONCENTRATIONS AS THE FUNCTION OF INITIAL CONCENTRATION AND DURATION OF INCUBATION. MAA AND CACODYLIC ACID: MEAN OF ALL LEVELS OF GLUCOSE.

	MAA				
Initial conc.	Days Incubation 0	3	7	15	28
10^2 ppm	100	84.3	85.5	86.0	67.6
10^3	100	98.2	68.6	88.3	87.6
10^4	100	108	69.8	67.0	78.0

	Cacodylic Acid				
Initial conc.	Days Incubation 0	4	10	14	31
10^2 ppm	100	110.4	84.0	163.4	86.4
10^3	100	92.8	92.8	118.8	97.0
10^4	100	91.6	111.5	52.0	84.8

TABLE 14.--ARSENIC REMAINING (MEAN OF LAST TWO OBSERVATIONS) AS THE FUNCTIONS OF INITIAL CONCENTRATIONS OF ARSENIC AND GLUCOSE. EXPRESSED AS PERCENT REMAINING AND MILLIGRAMS LOSS.

As ppm	MAA Initial glucose - percent 0.3	1.0	3.0	10.0
10^2	81.5 (-.185 mg)	77.7 (-.223)	62.5 (-.375)	87.7 (-.123)
10^3	105.2 (+.52)	121.3 (+2.13)	105.6 (+.56)	80.1 (-1.99)
10^4	59.6 (-40.4)	63.5 (-36.5)	73.9 (-26.1)	70.6 (-29.4)

As ppm	Cacodylic Acid Initial glucose - percent 0.3	1.0	3.0	10.0
10^2	124.1 (+.24 mg)	108.7 (+.087)	126.5 (+.265)	140.3 (+.403)
10^3	84.1 (-1.59)	101.9 (+.19)	121.9 (+2.19)	123.6 (+2.36)
10^4	68.9 (-31.1)	60.0 (-40.0)	69.2 (-30.8)	78.5 (-21.5)

This study demonstrates that high concentrations of organic arsenicals, especially MAA, are subject to attack by molds, and perhaps other microorganisms. Gross changes in arsenic content occurred within two weeks of incubation, with changes minimal during the first three days of colonization. The relation between energy supply and rate of reduction was not demonstrated, although this relation must exist. Presumably, energy becomes limiting at lower levels of glucose than those examined here.

The disappearance of substantial amounts of arsenic during an experimental procedure in which there were no opportunities for loss is conclusive evidence for loss by volatilization. Based on boiling points, the (BP 52.7°C. for trimethyl arsine) (cacodyl and cacodyl oxide have BP greater than 150°C) the conclusion is drawn that the arsines are the primcipal metabolite responsible for escape of arsenic from these cultures.

Field studies are concerned with the distribution of arsenic in various parts of the tree and the amount of arsenic reaching the soil after treatment. Cacodylic acid or MSMA were applied in the spring and fall at the following 4 locations:

1. Frater Lake, Washington, Lodgepole-pine type
2. 12 Mile Creek, Washington, Mixed conifer type
3. Pringle Falls, Oregon, Ponderosa pine type
4. Mt. June, Oregon, Douglas-fir type

The hypo hatchet was used to make applications at Pringle Falls and Mt. June while the hack-squirt technique was used at Frater Lake and 12 Mile Creek. Spring applications were made in May of 1971 while fall applications were made in November of 1971. Soil and forest floor material, and needles and twigs from the various crown positions were collected at each study site in the spring of 1972 and the fall of 1972. Results of analysis of these materials for arsenic are in Tables 15 and 16.

Considerable data analysis remains to be done. Based on the averages, however, it appears spring treatment resulted in higher concentrations of arsenic in twigs and needles than did fall treatment. Field observation of the levels of crown kills support this observation. Arsenic apparently moves rapidly from the point of injection to the most active metabolic sites in the foliage and stem tips in the spring season. Lower arsenic concentrations in fall vs. spring collections of twigs and needles may reflect the longer time between average treatment time and fall and spring collection dates. There is no great difference between the concentration of either MSMA or cacodylic acid in the needles and twigs or forest floor and soil. There is some elevation of arsenic in forest floor and soil at all study sites. The results from the Frater Lake and 12 Mile Creek sites are similar to those reported for estern Washington by Canutt and Norris. The Mt. June site has a naturally high level of arsenic in the forest floor and soil. Interestingly enough, in only one instance did the average concentrations of arsenic in forest floor and soil from any other study site exceed the background level of arsenic in forest floor and soil from the Mt. June study area.

A mathematical model to describe the results of this study is in preparation. When final analysis of data have been completed and the model prepared a supplement to this report will be issued.

Table 15.--Arsenic in forest floor and soil and in needles and twigs of conifers in stands treated with MSMA or cacodylic acid in May or November, 1971.

Treatment Area and Forest Type	Treatment	Arsenic (ppm)		
		Spring, 1972	Fall, 1972	
		Needles and Twigs[1/]	Needles and Twigs[1/]	Forest Floor and Soil[2/]
Frater Lake, Washington Lodgepole Pine	Control	1.0	2.0	2.5
	Cacodylic Acid, Spring	70.7	56.2	4.5
	Cacodylic Acid, Fall	28.8	26.7	9.7
	MSMA, Spring	59.1	60.1	3.7
	MSMA, Fall	46.7	49.8	3.8
12 Mile Creek, Washington Mixed Conifer	Control	5.8	2.1	1.6
	Cacodylic Acid, Spring	41.8	42.8	3.3
	Cacodylic Acid, Fall	31.2	23.7	3.0
	MSMA, Spring	59.7	65.8	4.8
	MSMA, Fall	36.7	47.0	4.7
Pringle Falls, Oregon Ponderosa Pine	Control	--	2.2	0.8
	Cacodylic Acid, Spring	59.3	28.1	1.3
	Cacodylic Acid, Fall	53.9	33.2	2.4
	MSMA, Spring	45.9	45.7	2.5
	MSMA, Fall	31.3	36.5	3.1
Mt. June, Oregon Douglas-fir	Control	--	4.5	6.8
	Cacodylic Acid, Spring	38.3	39.5	3.7
	Cacodylic Acid, Fall	44.0	37.3	12.5
	MSMA, Spring	39.8	15.8	6.9
	MSMA, Fall	8.4	18.3	8.9

1/ Each value is average of 7 observations at different positions in the crown.

2/ Each values is for a composite sample made from 50 M forest floor and soil core samples collected at random in treated plots.

Table 16.--Summary of arsenic in forest floor and soil and in needles and twigs of conifers in stands treated with MSMA or cacodylic acid in May or November, 1971

	Average Arsenic (ppm)	
	Needles and Twigs	Forest Floor and soil 1/
Spring treatment	48.0	3.8
Fall treatment	34.6	6.0
Spring collection	43.6	--
Fall collection	39.2	4.9
MSMA	41.7	4.8
Cacodylic acid	41.0	5.0
Frater Lake 2/	49.8	5.4
12 Mile Creek 2/	43.6	4.0
Pringle Falls 2/	41.7	2.3
Mt. June 2/	30.2	8.0
All treatments	41.3	4.9

1/ Fall, 1972 collection data only.

2/ Does not include control values.

SUMMARY OF STUDIES OF ARSENIC

BEHAVIOR IN THE FOREST

These studies established several important points about arsenic behavior in the forest after precommercial thinning with MSMA and cacodylic acid. Norris found both MSMA and cacodylic acid are highly mobile in the forest floor. In undisturbed cores of forest floor material, one inch of rain moved large quantities of both MSMA and cacodylic acid at least one inch. Thus, relatively little rain is required to move chemical which is spattered or spilled on the surface of the forest floor down into the forest floor and thereby reduce its availability to animals. Neither MSMA nor cacodylic acid are particularly mobile in soil and their careful use will not threaten ground water quality.

Canutt and Norris found small increases in arsenic in vegetation under trees treated with MSMA but the concentrations generally declined with time and distance from the tree. Nearly all the residues were within FDA established tolerances for cacodylic acid and MSMA in cottonseed hulls to be used for cattle feed. Arsenic residues in forest floor increased slightly at Colville and Lake Wenatchee but the levels declined with time and distance from the tree. Arsenic residues reached much higher levels under treated trees at Ellensburg but the residues declined fairly rapidly with time. Residues at Ellensburg were magnified because the site had been scarified prior to chemical treatment and no old forest floor was present to "dilute" fresh fall litter from treated trees. Small increases in soil arsenic levels also occurred. In general, each treated tree contributed to the arsenic level in nearby plants, forest floor and soil, but the arsenic levels remained low and in many cases showed a decrease with time after treatment. Relatively little effect was found at two times the crown radius.

Newton found molds indigenous to the forest appear to be able to metabolize MSMA and cacodylic acid to volatile compounds which have the odor of arsine. If this same mechanism is operative in the forest it may represent an important route of arsenic loss from treated areas. Studies in treated stands are not yet complete but information currently available indicates arsenic in forest floor and soil are increased slightly after chemical thinning but in all but one case, the levels were less than the background arsenic level at the Mt. June study area which has a naturally elevated arsenic background level. Newton estimates arsenic input to the forest floor and soil will vary from 0.3 to 1.5 pounds arsenic per acre after typical chemical thinning projects.

ARSENIC RESIDUES AND THEIR IMPACT ON CATTLE, SMALL ANIMALS, AND MICROBES

Five studies are in this section; two deal with arsenic residues in cattle or wildlife from treated areas and three deal with arsenic distribution, pathology, and toxicology in rabbits and cattle.

Arsenic residues in Cattle Grazed in treated areas

Hugh C. Maycumber
Veterinarian
Tonasket, Washington

This study is concerned with arsenic residues which may occur in cattle grazed in treated areas in Eastern Washington. Arsenic accumulation in hair is one of the classical indicators of arsenic exposure Arsenic in the hair of cattle before and after a summer's grazing in 2 treated areas were used as an index to arsenic exposure.

The Mt. Hull study area on the Okanogan National Forest was thinned with Glow-on tree killer and Silvisar 510 and is the site of cattle deaths in the summer of 1969. Hair samples were collected from 37 head of cattle on June 26, 1970, before the animals were turned out to graze for the summer approximately one year after the area had been thinned. A second collection of hair samples was made on October 14, 1970.

Hair samples were taken from 28 cattle on May 29, 1970, before they were turned out to graze in the Mill Creek study area on the Colville National Forest. This area was thinned with Silvisar 550 during the summer of 1970. On September 9, 1970, a second collection of hair samples from the same animals was made. The cattle were in the Mill Creek study area during the thinning operation. At the end of the grazing period, tissues from two of these cattle were analyzed for arsenic residues.

Results for both study areas are in Table 17. The data were arsenic subjected to one-way analysis of variance. The increase in arsenic concentrations between spring and fall samples is statistically significant at the 5 percent level at both sites. There was no statistically significant difference between study sites in samples collected at a given time.

These results indicate the increase in arsenic concentration in cattle hair during the summer is real. Arsenic is part of every environment, however, and the spring 1970 arsenic levels clearly indicate these animals had ingested arsenic previously (probably regularly). The literature suggests concentrations of arsenic in hair in the range of 0.03 to 1 ppm are in the normal concentration range. In many places, the concentration of arsenic in hair will be considerably higher than 1 ppm depending on the normal occurrence of arsenic in that environment. Inadequate holding facilities prevented a determination of the increase in the arsenic in hair from cattle grazed in nearby untreated areas.

Detectable residues of arsenic were not found in any body parts other than the hair from the two animals from the Mill Creek study area (Table 18). One of the animals had an increase in arsenic concentrations in hair which was 3 times greater than the average for all animals in the Mill Creek area, but no detectable residues of arsenic were found in the

Table 17.--Arsenic residues in cattle hair from forest areas thinned with MSMA or cacodylic acid.

Mt. Hull Study Area[1]			Mill Creek Study Area[2]		
Animal Number	Spring 1970	Fall 1970	Animal Number	Spring 1970	Fall 1970
	Arsenic (ppm)			Arsenic (ppm)	
20	0.56	0.33	23	0.18	0.44
42	0.39	0.65	71	0.20	0.27
43	0.47	0.74	92	0.24	0.61
44	0.96	1.20	25	0.23	0.30
46	0.18	0.56	59	0.48	0.98
47	1.28	0.81	26	0.25	0.45
48	1.04	1.53	157	0.64	0.59
49	0.27	1.14	27	0.39	0.94
50	0.73	1.07	28	0.21	0.90
51	0.41	1.28	29	0.24	0.35
52	0.35	0.77	15	0.58	1.02
53	0.69	0.62	84	0.60	0.82
54	0.54	1.04	183	0.26	1.18
55	0.31	0.72	31	0.23	0.79
56	0.44	0.83	142	0.58	0.58
57	0.31	0.50	35	0.97	0.50
58	0.23	0.97	189	1.28	1.08
59	0.53	0.68	32	0.73	1.15
60	0.49	1.09	33	0.38	0.53
61	0.35	0.81	63	0.66	0.98
62	0.88	1.15	58[4]	0.44	0.42
63	0.15	0.48	34	0.94	0.57
64	0.19	0.57	35	0.68	0.81
65	0.33	0.36	37	0.40	0.85
66	0.35	0.47	38	0.26	0.43
67	0.12	0.23	39	0.15	0.53
68	0.58	0.80	40	0.99	1.16
69	0.62	0.65	85[4]	0.57	1.30
70	0.10	0.32			
71	0.66	0.49			
72	0.28	0.57			
73	0.06	0.74			
74	0.71	0.82			
76	0.58	0.60			
77	0.27	0.34			
78	0.37	0.81			
79	0.17	0.49			
Average Values[5]	0.46 (a)	0.74 (b)		0.49 (a)	0.73 (b)

1/ Thinned with Glowon tree killer and Silvisar 510, 1969.
2/ Thinned with Silvisar 550, 1970.
3/ Arsenic determined by neutron activation, Washington State University.
4/ Animal slaughtered for tissue arsenic residue analysis, fall 1970.
5/ Average values with the same letter are not significantly different at the 5 percent level.

Table 18.--Arsenic residues in tissues from two cows and one female deer which were in the Mill Creek study area during thinning operations[1/]

Tissue	Arsenic (ppm)		
	Animal 58	Animal 85	Deer
Hair	ND[2/]	1.3	0.3
Blood	ND	ND	---
Urine	Insufficient sample	ND	---
Liver	ND	ND	ND
Kidney	ND	ND	ND
Lung	ND	ND	ND
Heart	ND	ND	ND
Tongue	ND	ND	ND
Spleen	ND	ND	ND
Muscle	ND	ND	ND
Brain	ND	ND	---
Paunch contents	ND	ND	ND
Udder	ND	ND	---
Droppings	--	---	ND

1/ The area was thinned with silvisar 550 during the summer of 1970. Cows were slaughtered in the fall of 1970 and tissues analyzed by the Wenatchee Pesticide Laboratory.

2/ ND - Nondetectable means less than 0.20 ppm arsenic.

body tissues. A female deer collected in the Mill Creek area in 1970 was also sampled for arsenic residues. Arsenic concentrations in hair of 0.26 ppm indicates this animal received limited exposure to arsenic. No detectable arsenic residues, however, were found in several body parts.

It appears the cattle in Dr. Maycumber's study were not exposed to significantly greater than normal amounts of arsenic as a result of their grazing in thinned areas. Lack of detectable arsenic residues in body tissues of cattle and deer suggest that grazing in thinned areas where carefully controlled applications of these chemicals are made may not lead to illegal arsenic residues in body tissues. FDA has established tolerances of 1 ppm arsenic in kidney and liver and 0.5 ppm arsenic in meat, fat and meat by-products of cattle for cacodylic acid (Federal Register, January 18, 1972). There is no established tolerance for MSMA because feeding cottonseed hulls containing 0.5 ppm arsenic is not expected to produce arsenic residues in cattle tissues (Federal Register, December 3, 1970).

Arsenic Residues in Wildlife from Areas Treated With Organic Arsenic Silvicides

Thomas Schroedel, Coordinator
Washington State Department of Natural Resources
Olympia, Washington

With other cooperators from DNR, USFS and
financial assistance from Key Chemical Co.

Wildlife, which are resident in areas thinned with organic arsenicals, may be exposed to the chemical through their consumption of vegetation which contains elevated levels of arsenic or by licking spots where the chemical has been spilled or has run down the stems of treated trees. This study asks the following specific questions:

1. How much arsenic is in various species of wildlife collected from areas thinned with MSMA or cacodylic acid?
2. Does the level of arsenic differ for different times after treatment, chemicals used, geographical locations, or the species or tissue sampled?

The objectives of this study are to determine if arsenic residues are accumulating in wildlife populations indigenous to treatment areas and if various species of wildlife can be used as indicators of arsenic contamination in the forest.

In this study, animals were trapped by D.N.R. and U.S.F.S. personnel at various intervals after treatment and arsenic residues determine at the Wenatchee laboratory. Three treatment areas were located in western Washington and four in eastern Washington. Nearly 500 analyses for arsenic in whole organisms or in certain body tissues were completed under this study. Results are available for chipmunks, grouse, deer, ground squirrels, mice, hares, mountain beaver, shrews, porcupines, voles, and a few birds. These species can be grouped into 3 general classes based on arsenic residues found in their tissues.

Group 1. This group contains 12 birds, 1 deer, and 7 grouse from areas treated with either MSMA or cacodylic acid. Animals in this group generally did not contain data table arsenic residues. The single deer included with this group was recognized by applicators as a long-time resident of the Mill Creek study area used by Maycumber. His topathologic examination of tissues from this deer showed no arsenic induced lesions and chemical analysis revealed no arsenic residues (Table 18).

Group 2. The majority of the animals that were trapped in this study are in this group. Chipmunks, mice, shrew, voles trapped in MSMA or cacodylic acid treated areas in eastern or western Washington are included. These animals contained low levels of arsenic shortly after thinning commenced. About 50% of the animals had arsenic residues between 0.5 and 9.8 ppm arsenic, between 2 and 30 days following treatment. Most residues were less than 5 ppm. Few animals collected more than 30 days after treatment

contained detectable residues. A single ground squirrel collected one day after treatment contained arsenic residues which ranged from 17 to 30 ppm in various body parts, but all the rest of the ground squirrels contained arsenic residues similar to those in voles, shrews, mice, and chipmunks.

Group 3. A total of 11 dead snowshoe hares are in the third group. These animals were all found in one particular treatment area.

With the exception of mice and chipmunks, the data is too fragmentary to permit the evaluation of questions originally posed for this study. Some interesting comparisons can be made for mice and chipmunks, however. For this purpose, the data were grouped into 10-day exposure intervals.

Table 19 compares whole body arsenic residues in mice captured in eastern Washington in areas thinned with MSMA or cacodylic acid. The data suggests use of MSMA rather than cacodylic acid results in slightly higher arsenic residues in mice. The difference is greatest between 0 and 10 days after thinning. After that time the differences are small. The percent of observations which are not detectable indicates arsenic is not uniformly spread through the population although in general lower percent ND is associated with higher percent observations greater than 1, 3, or 5 ppm arsenic. Cacodylic acid arsenic residues reached a maximum between 11 and 20 days after treatment and then declined. MSMA arsenic residues reached higher levels with peak average concentrations occurring between 0 and 10 days after treatment. MSMA residues remained elevated for about 30 days and then declined markedly.

Data in Table 20 compares arsenic in mice exposed to MSMA in either eastern or western Washington. There are not nearly as many observations for mice from western Washington, but the pattern seems clear nonetheless. Mice captured in western Oregon have much lower residues of arsenic in their tissues than animals captured in eastern Washington. This difference is most marked between 10 and 30 days after the area has been treated. No arsenic residues were detected in 32 mice captured in western Washington more than 10 days after treatment.

A similar pattern appears if we compare whole body residues for all mice, chipmunks, voles, and shrews exposed to MSMA or cacodylic acid (Table 21). The difference between eastern and western Washington study areas is less pronounced but there still is a lower frequency of arsenic residues greater than 1 ppm and a much greater frequency of animals with no detectable arsenic residues in animals captured in western Washington.

Snowshoe hare deaths occurred in connection with operational thinning operations with MSMA on the Colville, Washington National Forest in 1970. Thinning started near Colville, Washington, in mid-June 1970. Thinning crews in three different areas did not observe either live or dead hares until July 30 when one hare which had been dead for several days was found. Thinning crews then started searching their thinning areas and six more dead hares were found in the following 3-week period. Most of the animals had been dead for only 1 or 2 days. Thinning was terminated at this time due to the forest fire situation and was not resumed until mid-October. Diligent searches in areas treated from October through January revealed many live hares but no dead ones until January 13, 1971, when one additional dead hare was found in a previously

TABLE 19: ARSENIC RESIDUES IN MICE FROM AREAS IN EASTERN WASHINGTON TREATED WITH EITHER MSMA OR CACODYLIC ACID

Days after Treatment	Chemical	Number of Observations	Arsenic Residues: Average (ppm)	Range (ppm)	% observations ND[1/]	%observations greater than 1 ppm	3 ppm	5 ppm
0-10	Cacodylic acid	4	0.14	ND-0.57	75	0	0	0
	MSMA	12	1.93	ND-7.40	16	50	16	16
11-20	Cacodylic acid	12	1.10	ND-4.70	33	42	17	0
	MSMA	8	1.44	ND-5.30	37	50	12	12
21-30	Cacodylic acid	16	0.89	ND-3.76	44	31	6	0
	MSMA	19	1.89	ND-9.83	42	37	21	16
31-70	Cacodylic acid	12	0.17	ND-0.91	75	0	0	0
	MSMA	8	0.24	ND-0.80	62	0	0	0

1/ ND is not detectable (less than 0.5 ppm).

TABLE 20: ARSENIC RESIDUES IN MICE FROM AREAS IN EASTERN OR WESTERN WASHINGTON TREATED WITH MSMA OR CACODYLIC ACID

Days after treatment	Area in Washington	Number of observations	Arsenic Residues					
			Average (ppm)	Range (ppm)	% observations ND[1/]	% observations greater than 1 ppm	3 ppm	5 ppm
0-10	Eastern	16	1.48	ND-7.40	50	50	18	18
	Western	17	0.69	ND-4.30	71	28	11	0
11-20	Eastern	20	1.24	ND-5.30	35	60	20	20
	Western	8	ND	ND	100	0	0	0
21-30	Eastern	35	1.43	ND-9.83	43	44	25	19
	Western	16	ND	ND	100	0	0	0
31-70	Eastern	24	0.20	ND-0.91	67	7	0	0
	Western	8	ND	ND	100	0	0	0

1/ ND is not detectable (less than 0.5 ppm).

Table 21.--Arsenic residues in small mammals[1/] from areas in eastern or western Washington treated with cacodylic acid or MSMA[2/]

Days after treatment	Area	Number of observations	Arsenic Residues					
			Average (ppm)	Range (ppm)	% observations ND[3/]	% observations greater than 1 ppm	% observations greater than 3 ppm	% observations greater than 5 ppm
0-10	Eastern	34	1.29	ND-7.4	35	8	2	1
	Western	23	0.69	ND-4.3	78	7	3	0
11-20	Eastern	44	1.27	ND-5.89	25	8	2	1
	Western	17	ND	ND	100	0	0	0
21-30	Eastern	59	1.40	ND-9.83	32	11	5	2
	Western	20	ND	ND	100	0	0	0
31-70	Eastern	53	0.43	ND-4.11	62	4	1	0
	Western	13	ND	ND	100	0	0	0

1/ Shrews, mice, chipmunks, and voles.

2/ Data combined for cacodylic acid and MSMA.

3/ ND is not detectable (less than 0.5 ppm).

thinned area. The hare was not collected at that time. Three dead hares found in a treated area near Colville in February 1971, were collected and submitted for determination of arsenic residues in their tissues (Table 22).

Samples of tissue from five hares which died in August 1970 were sent to Dr. Dickinson at Washington State University for pathological and microbiological studies. Dickinson reported all animals had experienced enough post mortem degeneration that a good necropsy study was not possible. He could find nothing that pointed toward any particular cause of death and suggested arsenic poisoning. High levels of arsenic in tissues from these hares clearly indicate arsenic poisoning. Data on arsenic levels in hares which died in February 1971 are not available. However, evidence of vomiting and diarrhea near the dead animals suggest they died in distress which suggests arsenic poisoning.

Personnel at Colville report that most, if not all, of the dead hares collected in 1970 were found within a few hundred yards of "wash areas", locations where crews disposed of remaining silvicide in their squirt cans at the end of the working day and where they washed their equipment and hands. The normal procedure was to empty the contents of squirt cans and all wash water on the ground. Severe damage to vegetation contacted by these washings suggests exposure to high concentrations of the silvicide. Samples of soil, forest floor material, grass, and forbs contained high levels of arsenic which suggests wash areas may have been the source of arsenic which led to hare mortality in August 1970 (Table 23).

When thinning was resumed in October, excess silvicide and wash water were disposed of at the ranger station to avoid exposure of animals in treated areas. Lack of hare mortality from October to January suggests this procedure was effective. Field supervisors at Colville noted spillage of significant amounts of silvicide in treatment areas where hare mortality was observed in February 1971. The relation of spillage of silvicide and hare mortality deserves close attention.

Only limited data is available on arsenic residues in hares trapped in 1970 in other treated stands. Two animals collected 2 and 42 days following treatment of one area in eastern Washington did not contain detectable arsenic residues. Five animals collected in western Washington 232 days after treatment of an area contained arsenic residues which were either extremely low or nondetectable.

Preliminary examination of available evidence suggests:

1. Arsenic poisoning is theprobable cause of hare mortality at Colville.
2. Exposure to arsenic may result from the method of handling silvicide and wash water left over at the end of the day and to excessive spillage of silvicide in treated areas.
3. Several hares have been collected which do not contain appreciable residues of arsenic.
4. The low arsenic levels in muscle tissue from hares which survived acute exposure to MSMA suggest that short-term suspension of thinning activities or closure of recently thinned areas in connection with hunting seasons may be adequate to protect hunters from exposure to excessive arsenic residues in the tissues of game animals.

TABLE 22: ARSENIC IN SNOWSHOE HARES FROM COLVILLE NATIONAL FOREST, 1970.

Animal Number	Area	Exposure time (days after area treated)	Arsenic (ppm) Kidney	Liver	Muscle	Hair	Stomach	Bone
D-135	Joshlin Meadow1/	control	0.09	---2/	0.11	0.13	-------	----
D-136	Joshlin Meadow1/	control	0.11	-----	0.15	0.18	-------	----
D-55 3/	Joshlin Meadow1/	2-7 days	27.	-----	0.15	0.28	-------	----
D-36 3/	Hartbauer Basin	2-6 days	160	250	81	170	530	----
D-64 3/	Hartbauer Basin	2-6 days	130	140	96	130	450	----
D-66 3/	Hanson Creek	1-3 days	150	200	52	120	320	----
D-75 3/	Hanson Creek	1 day	----	160	22	27	130	----
D-77 3/	Hartbauer Creek	1 day	----	200	45	16	200	----
89 3/	Hanson Creek	1 day	520	460	33	540	180	----
91	Hanson Creek	?	0.77	0.22	0.14	2.04	ND	----

1/ Also called Ginder Meadows in some reports.

2/ -- Not analyzed

3/ Dead when found

TABLE 23: ARSENIC IN SAMPLES OF VEGETATION, FOREST FLOOR AND SOIL FROM WASH AREAS, COLVILLE NATIONAL FOREST, 1970

Area	Arsenic (ppm)			
	Grass	Forbs	Forest Floor	Soil
Joshlin Meadow	-----[1/]	-----	940	1900
Hanson Creek	580	930	---	2200
	16	1400		
Ottenbacker	----	----	470	760

1/ Not analyzed

ARSENICAL TOXICITY TO SNOWSHOE HARES

Jim Evans
Bureau of Sport Fisheries and Wildlife
Olympia, Washington

A commercial silvicide, Silvisar 550 (6 lb/gal MSMA-monosodium methane arsonic acid) was believed to be responsible for deaths of several snowshoe hares in eastern Washington. Evans determined the LD_{50} of MSMA (in Silvisar 550) for snowshoe hare as 173 (97-306) mg/kg; well below the 700-1200 mg/kg LD_{50} of MSMA reported for rabbits, rats, and mice. Live and dead hares from the eastern Washington treatment area had arsenical residues ranging from 100-150 ppm. Established residues in meat is 1 ppm. Fearing possible hazards to hunters hunting silvicide-treated areas, a degradation study on snowshoe hare using Silvisar 550 was conducted. Samples were analyzed at the Wenatchee laboratory.

RESULTS

Lacking sufficient test animals, 2 control and 10 test animals were used in the arsenical degradation study. All test animals were gavaged with 75 mg/kg MSMA using a Silvisar 550-corn oil formulation. Except for the control animals, pairs of hares were to be killed at 4 hours and 1, 2, 3, and 4 weeks. One control animal and two test animals were killed 4 hours posttreatment. Unfortunately, six of the eight remaining test animals died prior to killing (five at 30-42 hours and one at 450-500 hours). One test animal was killed 192 hours post-treatment; another at 336 hours posttreatment. The remaining control animal was killed at 504 hours (table 24).

Neither control animal showed arsenic residues. Arsenic residues in test animals were (ppm arsenic):

Table 24: Arsenic In Snowshoe Hares After Single Oral Dose Of MSMA

Time After Exposure (hours)	Arsenic Residue (ppm)	Number Animals and Method of Death
4	22.62 to 26.49 kidneys 0.75 to 1.03 muscle	2 killed
30 to 42	12.95 to 31.64 kidneys 2.44 to 3.82 muscle	5 died
192 to 336	0.72 to 0.99 kidneys nondectable residue in muscle	2 killed
450 to 500	91.45 kidneys 12.1 muscle	1 died

There appeared to be a rapid excretion of arsenic, but the animal that died 450-500 hours posttreatment suggests residue hazards may still exist 3 or more weeks after MSMA intake.

The animals can be placed in two groups based on the levels of arsenic in their tissues. Animals which survived exposure to the chemical had much lower arsenic residues than those which died as a result of exposure to a similar dose. These findings are consistent with our understanding that the organic arsenicals are excreted fairly rapidly from mammals. The magnitude of arsenic residues in kidney and muscle tissue from dead hares collected in August 1970 in the Colville area suggest they received doses of arsenic in excess of the LD_{50} for MSMA reported by Evans.

FUNCTIONAL, HISTOLOGIC, AND RESIDUES EFFECTS OF MSMA IN RABBITS

James R. Harr and Jerry Exon
Environmental Health Sciences Center, Oregon State University
Corvallis, Oregon

The objective of this study is to provide a basis for assessing the significance of residues in wildlife and to determine the arsenic body burden-dose relationship with respect to diagnostic lesions and arsenic residues in rabbits. This study proposed to answer the following specific questions:

1. What is the arsenic budget in rabbits chronically exposed to sublethal levels of MSMA?
2. What tissue lesions are observed and what is their relationship to arsenic residues?

The detailed results of this study are in a manuscript proposed for publication (Appendix 5).

In this study, New Zealand white rabbits were divided into three groups according to different exposure regimes. One group was fed a diet containing 50 ppm MSMA (27.5 ppm arsenic) and two animals of each sex were killed after 2, 4, 7, 12, 17, 24, 36, and 52 weeks of exposure. In a second group, four males and four females were fed the MSMA diet for 12 weeks and then the control diet for an additional 5 or 13 weeks at which time animals were killed. A third group of eight males and eight females served as non-exposed controls.

Analysis of liver and kidney tissue from rabbits continuously exposed to the MSMA indicated the concentration of arsenic in these tissues increased rapidly during the first two weeks of exposure and then reached a plateau. Arsenic residues in hair and muscle accumulated slowly up to 12 weeks of exposure and then reached a plateau. Bones did not accumulate detectable amounts of arsenic until 17 weeks after exposure. Bone and hair accumulated greater concentrations of arsenic than liver, kidney, or muscle.

Feces and urine contained significant quantities of arsenic. Approximately 70 percent of the ingested arsenic was excreted, 54 percent in urine and 46 percent in feces. Rabbits removed from the MSMA containing ration after 12 weeks of exposure and fed the basal ration for an additional 5 weeks showed a marked decline in arsenic concentrations in the tissues analyzed.

There were relatively few histologic changes in kidney, muscle, and liver tissue of animals exposed to MSMA for up to 52 weeks. Results appear to indicate these rabbits were able to tolerate a relatively high level of exposure to MSMA for a prolonged period of time with relatively minor effects. Histologic lesions of diagnostic significance were not detected.

Analysis of liver, kidney, and stomach contents from offspring of rabbits fed MSMA for 9 months contained no detectable arsenic residues indicating arsenic is not transmitted from the mother to the young, either *in uteri* or from nursing while the female is exposed to MSMA in her diet.

These results indicate that arsenic residues in edible tissues such as liver, muscle, and kidney may be elevated as long as rabbits are consuming vegetation which contains residues of MSMA. On the

other hand, tissue residues fall rapidly once the exposure period is ended. There are no estabished tolerances for arsenic in liver, muscle, and kidney of rabbits exposed to MSMA. The tolerance for cacodylic acid in cattle is 1.0 ppm arsenic in the kidney and 0.5 ppm arsenic in meat fat and meat byproducts.

Table 3 in Appendix 5 shows rabbits fed a diet containing 50 ppm MSMA continuously for 52 weeks did not develop residues of arsenic in the liver which exceeded the cacodylic acid tolerance for cattle. One sample of muscle after 12 weeks of exposure and one sample of kidney after 17 weeks did exceed the tolerance. Data from Canutt and Norris' study indicate residues of arsenic in vegetation under treated trees will be far below the 27.5 ppm arsenic exposure level used in this study.

CACODYLIC ACID AND MONOSODIUM ACID METHANEARSONATE TOXICITY STUDIES IN CATTLE

John O. Dickinson
College of Veterinary Medicine, Washington State University
Pullman, Washington

The purpose of this study was to produce acute and chronic cacodylic acid and monosodium acid methanearsonate toxicity in cattle. Both gross and microscopic pathology were to be considered as well as arsenic accumulation in various tissues. Part of the results from this study have been published (Appendix 6).

Two preparations of MSMA were utilized. One product contained 45% MSMA plus other ingredients, primarily a dye (Glowon Tree Killer, Key Chemicals, Inc.); the other solution contained only technical grade MSMA in 59% aqueous solution (Ansul 170 HC, the Ansul Co.). A 50% cacodylic acid solution (Silvisar 510, The Ansul Co.) was employed. In all instances the material was administered to cattle orally in gelatin capsules.

A rather low beginning dosage level was selected in an effort to gain information concerning chronic toxicity of the silvicides. Cattle were treated daily at a level of 10 mg/kg/day. Control hair samples were analyzed by neutron activation analysis (Nuclear Radiation Center, Washington State University) and also by a spectrophotometric method (Wenatchee laboratory) prior to beginning of treatment period. The dosage level of 10 mg/kg/day was continued for 3 weeks; at the end of this period a liver biopsy sample was taken for arsenic determination. A second three week period followed with a doubling of the dosage to 20 mg/kg/day with removal of a liver biopsy sample at the conclusion of this segment. A third 3 week period was planned to complete the chronic study; the dosage was to be increased to 30 mg/kg/day during the final period. At the conclusion of the program, all animals were necropsied and tissues were examined for gross and microscopic lesions of significance. Hair and tissue samples from all treated animals were assayed for arsenic content.

The procedure as outlined was not carried to completion; the cattle were unable to tolerate the MSMA solution(containing dye) at the lowest dosage rate for more than 10 days. At this point one steer (No. 3) died and treatment was stopped on the other two animals. All three had developed a rather severe diarrhea by the seventh day of treatment with a progressive dehydration and weight loss. Steer No. 5 became more lethargic and was unable to rise by day 12. This animal was killed with an overdose of pentobarbital sodium. Heifer No. 4 showed signs of recovery by this time and was observed daily following cessation of treatment. The animal made an apparent complete recovery.

Evidence of gross pathologic changes were present in kidney and abomasum of steers No. 3 and No. 5. Both kidneys were markedly swollen. On cross section the medulla appeared darkened, even hemorrhagic, whereas little change was noted in the cortex. There was a hemorrhagic castritis characterized by mucosal erosion and ulceration and intense hyperemia. A great deal of free blood was admixed with abomasal contents.

Microscopic examination of tissues from both steers indicated a marked hemorrhagic gastritis. Multiple foci of coagulation necrosis

having a midzonal distribution was present in the liver of steer No. 3. No significant lesions were found in liver or brain of steer No. 5; however, there was evidence of a marked diffuse nephrosis in both cases. It was concluded that renal tubular degeneration was the cause of death in the case of the first steer and would have been the ultimate cause of death in the second case.

Tissue arsenic levels are in Table 25 for both steers that died; results from both the neutron activation analysis as well as spectrophotometric method are recorded. Agreement between the two methods was quite staisfactory. A liver biopsy sample was withdrawn from heifer No.4 on day 10; the liver arsenic content was 7.2 ppm.

In animal No. 5, the kidney arsenic level dropped to a value below that of the liver. It is assumed that the animal was able to remove arsenic from the kidney rather rapidly. In spite of this, toxic nephrosis was the apparent cause of death. The phenomenon could be of diagnostic significance since pentavalent arsenicals are assumed to produce higher kidney tissue levels than liver tissue levels in poisonings. It would appear that cattle could conceivably deplete that arsenic content in the kidney rather rapidly to a value below that of the liver, and perhaps below diagnostic significance, but still succumb to irreversible tubular damage.

Steer No. 3 and steer No. 5 received a total dose of 100 mg MSMA per kg body weight. In the case of steer No. 3 (weight 475 lbs) this amounted to 21.6 g MSMA; steer No. 5 (weight 390 lbs) received 17.7 g.

The procedure was repeated using an aqueous solution of MSMA with the same dosage rate (10 mg/kg/day). Steer No. 12 died on the eighth day of treatment. Heifer No. 15 survived until day 16 even though treatment was discontinued on the eighth day. Both animals received a total dosage of 80 mg MSMA per kg body weight. Heifer No. 15 (weight 465 lbs) received 16.9 g and steer No. 12 (weight 260 lbs) 914 g MSMA during the treatment period of 8 days. Gross and microscopic pathologic lesions were quite similar to those described above. The tissue arsenic values for liver and kidney are in table 26. There was no apparent difference in the lethality of the aqueous MSMA solution and that containing the dye and other inert ingredients.

Cacodylic acid proved to be less toxic to cattle. Results are in Table 27. The two steers and one heifer survived the first three week period (10 mg/kg/day dosage rate) with minimal difficulty; all three exhibited a rather persistent diarrhea by the end of the second week but appetite was unimpaired. At the end of the fifth week heifer No. 1 exhibited a pronounced anorexia and weight loss; administration of cacodylic acid was discontinued at this point. This heifer ultimately recovered completely from the effects of the silvicide.

Steers No. 2 and 6 were judged to be in such poor physical condition at the end of six weeks (3 weeks at 10 mg/kg/day plus 3 weeks at 20 mg/kg/day) that both were euthanized at this point and tissues removed for arsenic determination and histopathologic examination. Tissue arsenic content (Table 27) was less than that acheived with MSMA administration even though the treatment period was longer. Pathologic changes, however, were quite similar. Acute enteritis, hepatic and renal degeneration were the primary changes observed. Cause of death, assuming animals had been permitted to die, would have been renal tubular degeneration. These steers each recieved a total dose of 630 mg cacodylic acid per

Table 25.--Tissue Arsenic Residues Following MSMA (44.9% Solution With Dye) Administration

	Steer No. 3			Steer No. 5			Control		
	Before	NA[1/]	S[2/]	Before	NA[1/]	S[2/]	Before	NA[1/]	S[2/]
Kidney	-	57.9 ppm	64.2 ppm	-	26.7 ppm	23.2 ppm	-	0.25 ppm	-
Liver	-	27.0	24.9	-	27.4	30.3	-	0.82	-
Brain	-	1.8	1.7	-	2.6	1.7	-	0.05	-
Bone	-	4.9	-	-	2.5	-	-	0.03	-
Muscle	-	10.3	-	-	7.4	-	-	0.02	-
Abomasum	-	-	-	-	14.2	17.7	-	0.02	-
Hair	2.7	3.3	-	1.1	1.4	-	0.82	0.81	-

[1/] Neutron Activation Analysis
[2/] Spectrophotometric Analysis

Table 26.--Tissue Residues Following Aqueous MSMA (59%)

	Steer No. 12	Heifer No. 15
Liver	17.7 ppm[1/]	1.6 ppm[1/]
Kidney	45.8	3.5

[1/] Spectrophotometric assay

Table 27.--Tissue Arsenic Concentration - Animals Receiving Silvisar 510 (50% Cacodylic Acid)

Tissue	Heifer No. 1	Steer No. 2	Steer No. 6
Kidney	-	5.554 ppm[1/]	11.940 ppm
Liver (beginning)	0.2271	0.179	0.082
(21 days)	3.090	3.711	2.492
(end)	-	4.236	3.177
Muscle	-	4.056	3.427
Abomasum	-	9.174	14.940
Brain	-	1.273	1.272
Bone	-	0.424	1.264
Hair (beginning)	2.040	0.980	1.010
(10 days)	4.270	2.555	2.041
(21 days)	6.144	5.099	2.488
(30 days)	6.617	6.985	5.752
(35 days)	19.220	7.752	10.630
(48 days)	32.850	15.720	12.930

[1/] Neutron Activation Analysis

kg of body weight. In the case of steer No. 2 (weight 460 lbs) this amounted to 131.5 g cacodylic acid; steer No. 6 (weight 520 lbs) received 148.7 g cacodylic acid.

Acute studies were conducted with both MSMA and cacodylic acid as a follow up to the chronic studies. In the acute studies cattle received a single oral dose of chemical. When cattle died or were killed tissues were examined for pathologic symptoms and arsenic residues. Results are in Table 28.

The results show again that MSMA is considerably more toxic to cattle than cacodylic acid. Animals which received lethal doses of MSMA had elevated levels of arsenic in both kidney and liver. Animals receiving cacodylic acid were still alive 14 days later and were sacrificed. Their kidney and liver arsenic levels were considerably lower. Arsenic levels were consistently higher in kidney than liver tissue.

Further chronic exposure tests with both MSMA and cacodylic acid were run to retest earlier findings on lethality of MSMA and to allow determination of blood urea nitrogen which is a measure of kidney function. Dosage regime and blood urea nitrogen determinations are in Table 29. Results confirm seven to eight doses of 10 mg/kg MSMA caused death of cattle. For one animal, six 15 mg/kg doses followed by four 30 mg/kg doses of cacodylic acid was lethal while a second animal received six doses of 12 mg/kg followed by five doses of 24 mg/kg without death occurring in the 14 days following the 29 day dosing period.

Blood urea nitrogen values in the range of 7 to 25 mg percent are normal. Values greater than this reflect the amount of kidney malfunction. Continued exposure to MSMA and to a lesser degree cacodylic acid caused an increase in blood urea nitrogen (Bun). However, there is also some evidence of return to normal levels when dosing is suspended at sublethal levels. For instance, steer 124 received MSMA through day seven and BUN exceeded the normal range. When dosing was stopped, BUN returned to normal level by day 19. Further insult to the kidney in the form of a resumption of dosing resulted in a second rise in BUN and the eventual death of the animal.

These results show clearly that kidney malfunction is involved in MSMA and cacodylic acid induced deaths in cattle. However, at least some kidney function is regained if exposure stops at sublethal levels.

TABLE 28: ACUTE TOXICITY OF MSMA AND CACODYLIC ACID IN CATTLE RECEIVING A SINGLE ORAL DOSE.

	Steer 47	Steer 23	Steer 34	Steer 17
MSMA[1/]	100 mg/kg	80 mg/kg		
Cacodylic Acid[2/]			600 mg/kg	800 mg/kg
Deaths	6 days post treatment	7 days post treatment	Killed 14 days post treatment	
Necropsy	Acute gastritis Toxic nephroses	Acute gastritis Toxic nephroses	Toxic nephroses	Toxic nephroses
Tissue	Arsenic (ppm)			
Liver	17.8	22.9	4.4	3.9
Kidney	44.1	33.8	7.1	8.4

1/ Ansar 170 HC, Ansul Co., Marinette, Wisconsin
2/ Silvasar 510, Ansul Co., Marinette, Wisconsin

TABLE 29: BLOOD UREA NITROGEN IN CATTLE CHRONICALLY EXPOSED TO MSMA AND CACODYLIC ACID

Days	Animal							
	Steer 124		Steer 130		Steer 131		Steer 128	
	MSMA (mg/kg)	BUN 1/ (mg%)	MSMA (mg/kg)	BUN 1/ (mg%)	CACODYLIC ACID (mg/kg)	BUN 1/ (mg%)	CACODYLIC ACID (mg/kg)	BUN 1/ (mg%)
1	10	10	10	8	15	16	12	22
3	10	17	10		15	17	12	20
5	10		10	22	15		12	
7	10	46		22	15	25	12	
9		40	10	21	15	24	12	27
10		30		20	15		12	
12		30		20		30		25
17		25		20		50		23
19		20		16				
21					30		24	
22	10	17	10	19	30	29	24	20
23					30		24	
26	10	50	10	22	30	60	24	20
28	10	103	10			60		25
29		187		45		Death	24	
30		Death	10	60				
32				173				18
33				Death				17
39								18
41								20
43								18

1/ Blood urea nitrogen

IMPACT OF MSMA AND CACODYLIC ACID ON MICROORGANISMS IN FOREST FLOOR AND SOIL

W. B. Bollen
Pacific Northwest Forest And Range Experiment Station
Corvallis, Oregon

Studies were also conducted to determine the toxicity of organic pentavalent arsenic compounds to microorganisms in forest floor and soil. These tests specifically determined: 1. The toxicity of cacodylic acid and MSMA to several common soil bacteria and molds in pure culture, and 2. The effect of these silvicides on the decomposition of organic matter by the native microbial populations in forest floor material and soil.

The first series of experiments included four bacteria (Bacillus subtillis, micrococcus caseolyticus, Enterobacter aerogenes, Pseudomonas fluorescens); two higher bacteria (Streptomyces antibioticus, Streptomyces olivaceus); and four molds (Penicillium claviforme, Penicillium restrictum, Aspergillus nidulans, Trichoderma viride). Bacteria were inoculated into triplicate sets of nutrient broth containing 0, 1, 10, 100, 1,000, or 10,000 ppm arsenic as cacodylic acid or MSMA. The higher bacteria and the molds were not tested in nutrient broth because their habit of growth produces no turbidity.

Additional tests with each organism were made using a paper disk assay where filter paper disks, 6 mm in diameter, were saturated with different concentrations of cacodylic acid or MSMA. Disks were placed on nutrient agar seeded with the test organisms. Nutrient broth cultures and agar plates were incubated at 28°C for 7 days and 4 weeks, respectively. Turbidity in nutrient broth was used as an index to growth of bacteria. The width of clear zones (zones of no growth) around each filter paper disk were measures of the toxicity to the organisms growing on the nutrient plates.

All the bacteria grew in liquid cultures with cacodylic acid and MSMA, although there was slight reduction in growth of Bacillus subtillis, and Micrococcus caseolyticus at 1,000 ppm arsenic.

In the paper disk assay on agar plates, all test organisms grew to the edge of the disks saturated with either cacodylic acid or MSMA at concentrations up to and including 10,000 ppm arsenic. These results indicate neither cacodylic acid nor MSMA are particularly detrimental to the growth of the bacteria, higher bacteria, and molds tested in this study.

The same soil and forest floor material which was used in the leaching studies reported by Norris, was also used to determine the impact of cacodylic acid and MSMA on carbon and nitrogen metabolism by native soil micro-flora. The chemical and biological characteristics of the forest floor material and soils are in Table 30.

Triplicate samples of the soil or forest floor materials were treated with MSMA or cacodylic acid to achieve concentrations of 0, 10, 100, or 1,000 ppm arsenic. Materials were placed in one pint bottles which were connected to a CO_2-free air manifold and each outlet air tube immersed in sodium hydroxide. The carbon evolved as carbon dioxide was trapped in the sodium hydroxide and was measured as an index to carbon metabolism. The bottles were incubated at 28°C, and carbon dioxide evolution determined 7, 14, 21, and 28 days after addition of the arsenic.

Table 30.--Chemical and biological characteristics of test materials[1/]

Test material	pH	Total carbon	Kjeldahl nitrogen	Molds		Bacteria	
				Thousands/g	Penicillia	Millions/g	Streptomyces
		------Percent-------			--Percent-		--Percent---
Soil							
Astoria	5.5	4.57	0.36	33	70	2.3	53
Edds	6.4	1.15	0.13	165	24	12.6	62
Klicker	6.7	3.30	0.27	65	17	2.3	60
Forest floor							
Douglas-fir	5.5	31.30	0.71	1,500	25	100.0	88
Mixed fir	5.5	37.90	0.77	3,560	68	15.8	94
Ponderosa pine	4.5	44.20	0.66	46	17	1.4	23

[1/] Methods of Bollen et al. (1967).

The rate of carbon dioxide evolution was much greater from forest floor than from soil (Tables 31 and 32). This undoubtedly reflects the generally higher carbon and microbial content of the forest floor. Statistical analysis revealed the rate of carbon dioxide evolution was significantly different among concentrations of arsenic for forest floor material treated with either herbicide, and for soil treated with MSMA. Cacodylic acid had no significant effect on the rate of carbon dioxide evolution from soil.

In forest floor material, the rate of carbon dioxide evolution declined with increasing concentrations of arsenic in both MSMA and cacodylic acid-treated materials. The decline was linear with increasing concentrations of MSMA and quadratic with increasing concentrations of cacodylic acid. MSMA caused an increase in the rate of carbon dioxide evolution from soil, which was linear with concentration.

The decomposition of organic matter and the release of nutrients is a particularly important function of microbes in the forest floor and soil. The total carbon dioxide evolved during the 28-day test period and the beginning carbon content of the test materials was used as a basis for calculating the percent decomposition of organic matter for each treatment (Table 33). Both cacodylic acid and MSMA altered the rate of organic matter decomposition in forest floor and soil. The greatest effects were at the highest concentrations of arsenic tested. However, even these effects were relatively small.

In other tests to determine the impact of these chemicals on nitrogen metabolism, 500 ppm nitrogen as peptone, was added to forest floor material or soil. Ammonia and nitrate nitrogen were determined at the time of peptone addition. Ammonification was determined 5 days later, 2nd nitrification 30 days later. After the addition of MSMA or cacodylic acid and the peptone, the bottles were closed with polyethylene film and incubated at 28°C. The results, expressed as percent ammonification and percent nitrification at 5 and 30 days of incubation, respectively, are in Table 34.

The test material had an intrinsically low rate of nitrification and little or no effect from the chemical was discernible. Ammonification was relatively rapid and with the exception of cacodylic acid in ponderosa pine forest floor material, neither cacodylic acid nor MSMA had an inhibitory effect on ammonification. In most instances, there was a slight to fairly large increase in ammonification in the presence of cacodylic acid or MSMA.

In terms of the use of these herbicides for pre-commercial thinning purposes, concentrations of arsenic greater than 10 ppm in forest floor and soil will occur infrequently and will then usually be restricted to a few square feet, if careful handling and application techniques are used. The impact of MSMA and cacodylic acid on the rate of organic matter decomposition or ammonification will decrease with time, as these compounds are metabolized, complexed, or otherwise reduced in bioactivity. It is concluded that neither cacodylic acid nor MSMA will seriously affect forest microbial populations, their decomposition of organic matter, or other functions important in the maintenance of soil fertility.

Table 31.--Evolution of carbon as carbon dioxide from forest floor material treated with cacodylic acid or MSMA

Mean mg C/50 g test material/week[1/]

Forest Floor	Arsenic (ppm)	CACODYLIC ACID WEEK 1	2	3	4	Total mg C	MSMA WEEK 1	2	3	4	Total mg C
Douglas-fir	0	260	188	125	105	678	258	181	143	126	708
	10	275	189	132	116	712	246	178	158	132	714
	100	224	169	134	104	631	233	170	144	116	663
	1000	234	140	94	78	546	226	164	135	92	617
Mixed-fir	0	355	201	184	129	869	300	194	156	148	798
	10	360	194	164	134	852	292	176	157	155	780
	100	325	186	169	127	807	292	184	158	115	749
	1000	328	189	136	105	758	283	186	131	121	721
Ponderosa pine	0	437	216	206	180	1045	363	191	202	179	935
	10	438	225	201	186	1050	363	202	181	168	914
	100	413	225	194	161	993	375	201	183	163	922
	1000	401	223	194	161	979	365	200	188	159	912

1/ Mean of three replications.

Table 32.--Evolution of carbon as CO_2 from soil treated with cacodylic acid or MSMA.

Mean mg C/50 grams test material/week[1/]

Soil	Arsenic (ppm)	CACODYLIC ACID WEEK 1	2	3	4	Total mg C	MSMA WEEK 1	2	3	4	Total mg C
Astoria	0	17	8	7	6	38	18	8	6	4	36
	10	16	8	5	6	35	18	8	6	5	37
	100	16	8	7	6	37	17	8	7	5	37
	1000	16	7	8	5	36	18	10	6	6	40
Edds	0	4	3	4	4	15	4	2	2	2	10
	10	4	3	3	3	13	3	3	2	2	10
	100	3	2	2	2	9	5	2	2	2	11
	1000	4	2	2	2	10	5	4	2	3	14
Klicker	0	8	4	3	4	19	8	3	2	3	16
	10	8	4	3	3	18	9	4	3	3	19
	100	8	4	3	3	18	8	6	3	3	20
	1000	7	3	2	3	15	10	5	3	3	21

1/ Mean of three replications

Table 33.--Decomposition of organic matter in forest floor and soil in 28 days at 28°C after addition of cacodylic acid or MSMA.

	Organic Matter Decomposition (%)							
	Cacodylic Acid (ppm As)				MSMA (ppm As)			
Forest Floor	0	10	100	1000	0	10	100	1000
Douglas-fir	4.33	4.54	4.03	3.49	4.52	4.56	4.24	3.94
Mixed fir	4.58	4.50	4.26	4.00	4.22	4.12	3.95	3.81
Ponderosa pine	4.71	4.76	4.49	4.42	4.23	4.21	4.18	4.13
Soil								
Astoria	1.66	1.53	1.62	1.58	1.62	1.62	1.58	1.84
Edds	2.61	2.26	1.57	2.09	1.74	1.74	1.91	2.43
Klicker	1.15	1.09	1.09	0.91	1.03	1.21	1.09	1.21

Table 34--Ammonification and nitrification at 28°C in forest floor material and soil containing MSMA or cacodylic acid.

Forest Floor	Arsenic (ppm)	Cacodylic Acid		MSMA	
		Ammonification (%)	Nitrification (%)	Ammonification (%)	Nitrification (%)
Douglas-fir	0	56	0.2	31	0.2
	10	63	0	46	0.4
	100	91	0.2	46	0.2
	1000	123	0.4	35	0.6
Mixed fir	0	85	0	70	0.4
	10	82	0	96	0
	100	103	0	98	0.2
	1000	110	1.0	98	0.6
Ponderosa pine	0	25	0	8	0.2
	10	21	0	7	0.2
	100	17	0	15	0.2
	1000	7	0	25	0.2
Soil					
Astoria	0	65	0	57	0.2
	10	73	0.2	63	0
	100	65	0.4	60	0
	1000	70	0	58	0
Edds	0	69	0	62	1.0
	10	76	0.4	69	0.4
	100	82	0.2	62	0
	1000	71	0	71	0
Klicker	0	71	1.8	62	0.8
	10	69	1.2	68	1.6
	100	67	1.4	60	0.4
	1000	62	0	71	0

SUMMARY OF STUDIES OF ARSENIC RESIDUES AND TOXICITY IN ANIMALS

Maycumber found cattle grazed in treated areas showed increased levels of arsenic in hair, but he had no measure of the normal increase expected because of native arsenic. The level or arsenic in hair at the end of the summer was within the normal range for arsenic in hair. There was no difference between arsenic levels in hair from cattle grazed in one area thinned 1 year earlier and cattle grazed in another area thinned during the study grazing season. This also lends support to the idea the end of season arsenic levels in hair represent "normal" arsenic intake levels. No arsenic residues were detected in several tissues from two cattle grazed in the area thinned during the grazing season.

Schroedel found arsenic residues in mice were the best indicator of the use of MSMA or cacodylic acid in thinning operations. Cacodylic acid seemed to produce lower whole body arsenic residues in mice than did MSMA but the body burden for both chemicals declined with time after exposure. The difference between MSMA and cacodylic acid arsenic residues in mice was not very great. Animals in western Washington had markedly lower body burdens of arsenic than animals in eastern Washington. No arsenic residues were detected more than 10 days after treatment in western Washington. Arsenic residues are either less available or less attractive in western Washington.

Careless handling and application of MSMA (and probably cacodylic acid) can result in snowshoe hare deaths. Evans reports the LD_{50} of MSMA is about 170 mg/kg in snowshoe hare. Arsenic residues in body tissues of hares which survived a single oral dose of MSMA declined fairly rapidly. Animals which succumbed to the chemical contained very high arsenic residues in kidney, liver, and muscle.

Harr and associates fed 50 ppm MSMA to rabbits for various intervals. They did not find lesions of diagnostic significance. Tissue arsenic levels were related to exposure and declined rapidly when the animals were fed a ration which did not contain MSMA. Arsenic was not transmitted to offspring either *in uteri* or in milk from females exposed to MSMA in their diet.

Dickinson reported MSMA was considerably more toxic to cattle than previously suspected. Oral doses of 10 mg/kg/day for about 10 days resulted in the death of two of three test animals. Toxic nephrosis in the kidney was the cause of death and may be of diagnostic significance in suspected MSMA poisonings in cattle. Cacodylic acid was considerably less toxic than MSMA. Tissue arsenic levels were lower in cacodylic acid-exposed animals than in those receiving MSMA but the pathologic symptoms were similar. The toxicity of MSMA (and to a lesser degree cacodylic acid) will require extra care during handling and application of the silvicides in areas that are or will be grazed in the near future.

Bollen found neither MSMA nor cacodylic acid at field concentrations had a significant effect on the growth and survival of bacteria and fungi in pure culture. There was little or no impact on carbon or nitrogen metabolism. The careful use of MSMA and cacodylic acid will not have an adverse effect on forest microorganisms or their activities important in the maintenance of soil fertility.

OPERATIONAL LEVEL THINNING OPERATIONS

STUDIES OF EXPOSURE OF APPLICATORS AND ANIMALS TO ARSENICALS

Logan A. Norris
Pacific Northwest Forest And Range Experiment Station
Corvallis, Oregon

After the results of the 1970 field season studies were available, it appeared it was possible to use the arsenicals in a safe manner, at least on a limited scale. During the summer of 1971, several tests were conducted to determine if this work could be conducted safely at the operational level. Safe application requires minimum exposure to both applicators and animals. The arsenic content of urine from applicators was used as a measure of human exposure and arsenic residues in mice collected in treated area served as an indicator of their exposure to the herbicide.

Individual five-man Forest Service thinning crews, and the areas they operated in were study units. One study unit each was located on the Siuslaw and Kaniksu National Forests, and three units were on the Colville National Forest. General guidelines as to the type of application equipment, protective gear, and methods of handling and applying the herbicides to minimize exposure to the applicator and small mammals were issued to each crew. Variations in the type and density of vegetation, roughness of terrain, and climatic factors required the use of methods and protective equipment particularly suited to the circumstance. No attempt to standardize techniques among crews was made. Specific guidelines developed by the Colville and Siuslaw National Forests are in Appendix 8.

During the same period, the Washington State Department of Natural Resources conducted chemical thinning operations using young forest workers. A ten-man crew from the Cedar Creek Youth Camp and a nine-man crew from Naselle Youth Camp were involved in these operations.

Individual urine samples were collected from each applicator in each study area every Friday at the end of the work-day, and on every third Monday, before the start of the work-day, throughout the thinning season. Samples from at least five persons not exposed to the chemical served as controls. Individual urine samples were analyzed by the Wenatchee laboratory, and the concentration of arsenic in urine and the osmolality of the urine determined.

Mice were chosen as indicators of the exposure of small animals to the organic arsenicals. Each crew and its operating area were considered as individual study units. Specific monitoring areas were established within study units. A monitoring area was defined as the contiguous area treated by the crew on three consecutive working days. A different monitoring area was established once every three weeks for each thinning crew during the whole study period. Within a given monitoring area, mice were collected approximately 1, 5, 15, and 30 days after the area was treated. This sampling schedule was repeated in each of the monitoring areas which are established during the study period. Mice from areas with no previous chemical treatment history served as controls. Earlier studies had shown 97% of the mice from untreated areas contained no detectable quantities of arsenic. Mice (whole bodies) were analyzed at the Wenatchee laboratory for arsenic content and the results expressed as ppm arsenic.

Colville National Forest

The most complete data on exposure of applicators and animals was from two thinning crews on the Colville National Forest. Three study units were completed. Study unit 1 consisted of five men which applied Glowon herbicide from June 6, 1971 through September 10, 1971. Study unit 2 was the same group of five men who applied Glowon, Glowon with TMTD or Silvisar 550 from October 25, 1971 through January 19, 1972. Study unit 4 consisted of five men applying Glowon with TMTD from July 6th through September 10, 1971. Results of urine-arsenic analysis are in Table 35.

These data demonstrate the Colville crews were successful in minimizing their personal exposure. There is no marked difference in the mean arsenic levels between Mondays and Fridays, and the percentage of observations greater than 0.3 ppm is rather small. The concentrations of arsenic in animals from the Colville National Forest are in Tables 36 and 37.

Data for mice and chipmunks indicate animals captured in treated areas have levels of arsenic which are higher than those captured in untreated areas. Arsenic residues appear to peak around five days after treatment and then decline slowly with time. For a given study unit, the average concentrations of arsenic appear to increase during the course of the treatment season. In the case of study unit 1, the average arsenic concentrations increased from 0.14 ppm in late June to 0.34 ppm in July and 0.73 ppm in August. Similar trends occurred in study unit 4.

The percentage of animals analyzed which contained non-detectable levels of arsenic indicates the distribution of arsenic throughout the population of mice and chipmunks and shrews is not uniform. In the case of study unit 1, 50 or more percent of the animals captured contained no detectable quantities of arsenic. In study unit 4 which was conducted by another crew at about the same time of year, a somewhat lower incidence of non-detectable residues in captured animals occurred, but on the average, more than 1/3 of the animals captured contained no detectable residues. Study unit 2, which was conducted during the winter months, had a high incidence of non-detectable residues; nearly 3/4 of the captured animals contained no detectable quantities of arsenic.

The average arsenic concentration in mice, chipmunks, and shrews was not greatly influenced by time after treatment (Table 36). Examination of Table 37 however, shows time after treatment had a marked effect on the number of observations which exceed 1 ppm.

Only a limited number of snowshoe hares were captured and this prevents presentation of residue data in the same format used for mice, shrews, and chipmunks. Table 37 shows live hares which were captured in treated areas contained higher concentrations of arsenic than hares from untreated areas (controls). The average concentrations are relatively low however, and do not exceed the FDA established tolerances for cacodylic acid in cattle.

The one dead hare found on the Colville National Forest contained elevated levels of arsenic in all tissues, and was clearly a victim of arsenic poisoning. This pattern of high residues in dead hares and relatively low levels in live hares has occurred in previous years. Hares in treated areas which appear to be normally active probably contain only low levels of arsenic in their tissues. Dead animals, those which

TABLE 35: ARSENIC IN URINE FROM COLVILLE NATIONAL FOREST THINNING CREWS, 1971 and 1972.

Study Unit, Chemical And Dates Monitored	Arsenic in Urine (ppm)			
	Mondays		Fridays	
	Mean	Range	Mean	Range
Study Unit 1				
5-man crew, Glowon				
6/6/71 - 9/10/71	0.06	ND[1/]-0.21	0.10	ND - 0.34
Observations greater than 0.3 ppm	0%		4%	
Study Unit 2				
5-man crew, Glowon, Glowon and TMTD, Silvisar 550				
10/25/71-1/19/72	0.04	0.01-0.21	0.06	0.01-0.32
Observations greater than 0.3 ppm	0%		2%	
Study Unit 4				
5-man crew, Glowon and TMTD				
7/6/71-9/10/71	0.03	0.01-0.11	0.05	0.01-0.13
Observations greater than 0.3 ppm	0%		0%	
Control Group				
6/6/71-1/19/72	0.02	ND-0.08	0.02	ND-0.18

1/ ND - not detectable (less than 0.01 ppm).

Table 36.--Arsenic in mice, chipmunks, and shrews, Colville National Forest, 1971 and 1972.

Arsenic ppm

Study Unit and Monitoring Area	Chemical	Date	Days Since Area Was Treated 1	5	15	30	Mean	% ND[1/]
1-1	Glowon	6/14/71-7/9/71	-[2/]	0.11	0.12	0.18	0.14	62
1-2	Glowon	7/13/71-8/6/71	-	0.04	0.58	0.36	0.34	50
1-3	Glowon	8/6/71-9/4/71	-	1.95	0.18	ND	0.73	50
1-4	Glowon	8/31/71-9/24/71		Not Complete				
	Arsenic,	MEAN ppm	-	0.70	0.29	0.12		
2-1, 2, 3 & 4[3/]	Glowon, Glowon and TMTD, Silvisar 550	10/29/71-2/7/72	0.50	ND	0.5	0.3	0.33	72
4-1	Glowon and TMTD	7/9/71-8/6/71	-	0.41	0	1.10	0.84	50
4-2	Glowon and TMTD	7/29/71-8/27/71	0.49	1.44	1.08	0.98	1.07	24
4-3	Glowon and TMTD	8/19/71-9/16/71	1.23	0.84	0.52	1.24	1.01	47
4-4	Glowon and TMTD	9/10/71-10/9/71	0.5	1.42	1.76	0.52	1.13	31
	Arsenic,	MEAN ppm	0.74	1.03	0.84	0.96		

1/ ND - not detectable (less than 0.5 ppm).

2/ - means insufficient data.

3/ Insufficient number of animals were captured in study unit 2. Data for all 4 monitoring areas were combined.

Table 37--Arsenic in mice, shrews, chipmunks, and in snowshoe hare, Colville National Forest, 1971 and 1972.

Combined Data - Areas 1 and 2, for mice, chipmunks, and shrews

Days After Treatment	% Observations greater than: 1 ppm	3 ppm	5 ppm	Number of Observations
1	20	0	0	10
5	17	4	0	24
15	8	0	0	24
30	4	0	0	23

Combined Data - Areas 1, 2, and 4, snowshoe hares

Exposed 1 - 30 days		Concentration Arsenic (ppm) Muscle	Liver	Kidney	Hair
Condition at Capture					
Live (14 animals)	MEAN	0.46	0.18	0.43	0.50
	RANGE	ND[1/] - 1.9	ND - 0.8	ND - 2.3	ND - 2.5
Dead (1 animal)		110	160	240	5.1
Unexposed					
Controls (3 animals)		ND	ND	ND	ND

1/ ND is not detectable (less than 0.5 ppm).

do not show a normal amount of activity, or those which appear to be in distress, may contain elevated levels of arsenic in their tissues and are not fit for human consumption.

Data from study units 1 and 4 are particularly interesting (Table 38). The rabbit repellant, TMTD was added to Glowon in study unit 4, but results in Table 38 indicate mice, shrews, and chipmunks may be more likely to pick up the Glowon-containing TMTD than Glowon alone. It is difficult to make a direct comparison between these two study units, however, because different crews were involved and they may not have used the same degree of care in application of the chemical. Examination of average urine-arsenic levels, however, suggest the crew in study unit 4 was very successful in minimizing their own personal exposure and presumably exposure of animals as well (Table 38).

The results of these studies on the Colville National Forest are highly satisfactory. The Colville crews have demonstrated it is possible to apply the organic arsenicals on an operational basis with minimum exposure to both applicator and animals.

Table 38.--Summary arsenic in urine from applicators and small animals, Colville National Forest, 1971 and 1972.

	Study Area and Chemical		
	1	2	4
	Glowon	Glowon, Glowon and TMTD and Silvisar 550	Glowon and TMTD
Mice, shrews, and Chipmunks			
% Observations ND[1/]	54	72	38
% Observations> 1 ppm	8.7	9.4	37.0
% Observations> 3 ppm	2.2	0	8.8
% Observations> 5 ppm	0	0	0
Mean Arsenic (ppm)	0.37	0.33	1.02
Unexposed Controls (8 animals) All Observations ND			
Applicators			
Mean Urine Arsenic (ppm)			
Mondays	0.06	0.04	0.03
Fridays	0.10	0.06	0.05
% Observations > 0.3 ppm	4.0	2.0	0

1/ ND is not detectable (less than 0.5 ppm).

Siuslaw National Forest

A five man team applied organic arsenicals in operational thinning on the Siuslaw National Forest. This crew was the subject of study by Dr. Sheldon Wagner. The results of his study have already been covered. Additional data from the Siuslaw National Forest are in Table 39.

Only low levels of arsenic were found in urine from applicators. No observation exceeded 0.3 ppm arsenic in urine. On this basis, this crew was highly successful in avoiding personal exposure to the silvicide. Residue levels in mice were extremely low, with a high percentage of animals containing no detectable residues. The Siuslaw crew was unsuccessful in trapping hares and substituted mountain beavers in their place. Arsenic residues in tissues from mountain beavers were higher in exposed animals than in controls. On the average, these values appear slightly higher than values for similar tissues in rabbits from treated areas on the Colville National Forest. The arsenic concentrations in mountain beavers collected five days after treatment were somewhat lower than levels in animals exposed for only a single day, suggesting fairly rapid excretion of ingested arsenic. In general, the Siuslaw National Forest crew was highly successful in their efforts to minimize exposure of both applicators and animals.

TABLE 39: ARSENIC IN URINE FROM APPLICATORS AND ANIMALS, SIUSLAW NATIONAL FOREST, 1971.

Arsenic In 24 Hour Urine Sample (ppm)

	Preexposure	7/9/71	7/16/71	7/24/71	7/30/71	8/6/71
Mean	0.03	0.04	0.08	0.11	0.10	0.09
Range	0.01-0.05	0.01-0.09	0.02-0.16	0.05-0.19	0.05-0.15	0.03-0.12

0% > 0.3 ppm

Arsenic In Mice (ppm)
(Data From 3 Monitoring Areas Combined)
Days After Treatment

	1	5	15	30
Mean	0.19	0.14	ND	0.04
Range	ND-0.8	ND-0.6	All ND	ND-0.5
Number of Animals	11	16	16	14
% ND[2/]	64	75	100	93

Unexposed control mice (9 animals) All ND.

Arsenic In Mountain Beaver (ppm)[3/]

Days After Treatment	Muscle	Liver	Kidney	Hair
0 (Unexposed controls)	ND	0.3	ND	0.4
1	1.0	1.5	0.8	1.2
5	0.3	0.4	0.8	0.6

1/ Silvisar 510 (cacodylic acid).

2/ ND is not detectable (less than 0.5 ppm).

3/ 2 animals each collection time.

Kaniksu National Forest

A five-man crew operated on the Kaniksu National Forest during the summer of 1971 and participated in the study to determine if organic arsenicals could be handled on an operational basis. This crew consistently ran into problems throughout the summer, and their use of the chemical was suspended near the end of July to prevent the continued high exposure of applicators. The use was reinstituted for one week, late in August. Urine-arsenic levels quickly returned to previous high levels. Results of urine-arsenic monitoring for the Kaniksu National Forest crew are in Tables 40 and 41.

The results indicate clearly this crew was not successful in avoiding personal exposure to the chemical. The mean concentration of arsenic in urine rose rapidly after the beginning of the thinning season and remained at a relatively high level through the first two weeks of July. After that time, concentrations rose rapidly and thinning was suspended on August 2, 1971. Arsenic levels in urine then dropped rapidly and had returned to background levels by August 20, 1971.

Thinning was resumed for four days, on August 23, 1971, but arsenic concentrations in urine quickly shot up. All three men on the crew exceeded the 0.3 ppm arsenic in urine level. The daily diary of the crew foreman shows numerous occasions (underlined) when applicators received considerable exposure to the chemical. Lack of use of appropriate gear was also a factor.

Relatively few animals were collected from the Kaniksu study areas. There is insufficient data to show changes in arsenic concentrations with time after exposure, or at various times through the treatment season, but the mean and range of arsenic levels are useful (Table 42). Average arsenic concentrations were somewhat higher than those encountered at either Colville or Siuslaw National Forests. Higher concentrations in animals are anticipated when applicator urine-arsenic levels are elevated.

Table40.--Arsenic in urine from applicators, Kaniksu National Forest, 1971[1/]

Date	Arsenic (ppm) Mean	Range	Crew Size	Number > 0.3 ppm	% > 0.3 ppm
June					
15	0.08	0.02-0.20	5	0	0
18	0.31	0.08-0.42	5	3	60
25	0.20	0.04-0.33	5	1	20
July					
2	0.21	0.05-0.33	6	2	33
6	0.17	0.03-0.48	6	1	17
9	0.15	0.07-0.37	6	1	17
16	0.29	0.05-0.68	6	2	33
23	0.72	0.19-1.32	6	4	67
26	0.53	0.11-1.01	6	5	83
30	0.49	0.10-1.38	6	3	50
August	Chemical thinning suspended 8/2/71				
6	0.17	0.13-0.36	6	1	17
13	0.02	0.02-0.03	6	0	0
20	0.02	0.01-0.03	5	0	0
	Thinning resumed 8/23/71				
27	0.85	0.32-1.74	3	3	100
	Last day of thinning 8/27/71				

1/ Silvisar 510 (cacodylic acid).

Table 41--Arsenic in urine from applicators, Kaniksu National Forest, 1971[1/]

Crew Member	Average Monday Level	Average Friday Level	% Friday Levels Above 0.3 ppm	Maximum Observed
1	0.11	.14	13	.36
2	.24	.43	75	1.12
3	.50	.58	71	1.38
4	.23	.43	29	1.32
5	.31	.50	62	1.74
6	.16	.20	20	.38

Unexposed Controls

6 men		6/29/71 - 8/27/71
	Mean	0.02
	Range	ND[2/] - 0.17

1/ Silvisar 510 (cacodylic acid).

2/ ND is not detectable (less than 0.01 ppm).

Table 42.--Arsenic in animals, Kaniksu National Forest, 1971.

Mice, chipmunks, and shrews	Arsenic (ppm)
Mean	2.2
Range	ND[1/] - 12.0
Number of animals	15

% Observations greater than:

1 ppm	3 ppm	5 ppm
50	25	15

Squirrels	Muscle	Liver	Kidney	Hair
Mean	2.6	11.7	10.5	2.2
Range	ND-5.6	5.6-14.2	4.9-15.6	1.17-2.75
Number of animals	4			

1/ ND is not detectable (less than 0.5 ppm).

Washington State Department Of Natural Resources

The Washington State Department of Natural Resources operated a ten-man and a nine-man thinning crew during the summer of 1971. Urine-arsenic levels were monitored following the same schedule used for the Forest Service crews. Results of the urine-arsenic monitoring are in Table 43.

The Washington Department of Natural Resources crews were moderately successful in minimizing exposure to applicators. The mean arsenic levels for samples collected on Fridays were comparable with those on the Colville National Forest, but the range of values was somewhat greater and the incidence of values exceeding 0.3 ppm was also greater. The level of exposure experienced by these applicators probably does not present a serious problem.

The results of the 1971 season test are very encouraging. Of the six crews which operated during that period, five were successful in minimizing exposure to applicators. Three out of four crews were successful in minimizing exposure to animals. These results indicate that use of proper protective gear, adequate training of crews, and effective field supervision are important factors in the safe handling and application of organic arsenicals in operational thinning programs.

Table 43--Arsenic in urine from applicators, Washington Department of Natural Resources, 1971.[1/]

Cedar Creek Youth Camp	Exposed Group
All Monday and Friday values during weeks with no exposure	
Mean 0.06 ppm	Range ND[2/] - 0.13
0% > 0.3 ppm	
Friday values during weeks of exposure	
Mean 0.08 ppm	Range ND - 0.52
10.2% > 0.3 ppm	
28.3 hours exposure per man per week	
Combined Monday and Friday values	Control Group
Mean 0.02 ppm	Range ND - 0.10
0% > 0.3 ppm	

Naselle Youth Camp	Exposed Group
Monday values during weeks of exposure	
Mean 0.03 ppm	Range ND - 0.08
0% > 0.3 ppm	
Friday values during weeks of exposure	
Mean 0.09 ppm	Range ND - 0.49
4.5% > 0.3 ppm	
Mean 17 hours exposure per man per week (average)	
	Control Group
Combined Monday and Friday values	
Mean 0.02 ppm	Range ND - 0.17
0% > 0.3 ppm	

1/ Glowon tree killer (MSMA)

2/ ND is not detectable (less than 0.01 ppm).

TRAINING FOR OPERATIONAL THINNING

John Hughes
Siuslaw National Forest
Corvallis, Oregon

Successful chemical thinning requires both an effective and a safe application of the chemical. A totally safe application which does not accomplish the objectives of the thinning program is not acceptable. Effective thinning which results in significant exposure of applicators and animals to the chemical is also not acceptable. Effective and safe chemical thinning requires adequate training of both the field supervisor and crew members.

The following questions need attention in training the field supervisor:

1. What are the scope and objectives of the program?
2. Who will plan and organize the various aspects of this program?
3. What persons are responsible for what parts of the program? Clearly establish the field supervisor as responsible for day-to-day conduct of the work and for following previously selected techniques of chemical handling and application.
4. Who is responsible for crew health and safety?
5. Who is responsible for equipment and protective gear, procurement, maintenance and replacement?
6. What guidelines are appropriate for the safe and effective operation of the thinning program?
7. What are the training needs of crew members and how will training be conducted and by whom?
8. What hazards do the chemical pose to applicators and animals and what is on the manufacturers' label?
9. What levels of quantity and quality of thinning production are required?
10. What types of records and reports are required and who is resopnsible for their preparation and review?
11. What are indicators of poor handling and application techniques and what steps are to be taken if they appear?

The crew members need orientation to the following items:

1. Selection of crop trees.
2. Familiarization with the chemical (read and discuss the information on the manufacturer's label).
3. Familiarization and discussion of guidelines for operational thinning.
4. Application equipment use, maintenance, and replacement.
5. Protective equipment use, maintenance, and replacement procedures.
6. Personal cleanliness.
7. Requirements of the monitoring program.
8. Indicators of unacceptable handling and application techniques.
9. Safety training with regard to chemical thinning tools, the chemical, and other items as appropriate.
10. Clear designation of the authority of the field supervisor to require changes in personal practices which are not acceptable.

A combination of methods to accomplish training of field supervisors and crew members may be used. Field supervisors who are inexperienced in chemical thinning may require a few days orientation with an experienced crew. And must have the same training given to crew members. Other training can be accomplished by conference and directed reading.

Crew members should be trained by professional staff or field supervisors experienced in chemical thinning programs. A review and discussion of guidelines for operational thinning are required.

Periodic inspection of field operations by professional staff is necessary. Critique for the field supervisor alone and for the supervisor and the crew together are essential to insure adherence to the guidelines. Experience has shown repeatedly that strong and effective leadership of work crews by a field supervisor and rigorous training program for supervisors and crews result in both an effective and safe chemical operation.

GUIDELINES FOR OPERATIONAL THINNING WITH ARSENICALS

John Lyman
Colville National Forest
Colville, Washington

This section considers the following subjects which were developed by a panel at the arsenic review conference in Portland, Oregon, November 27, 1972:

A. Selection of Chemicals
B. Packaging
C. Application
D. Protective Equipment
E. Special Crew Precautions
F. Chemical Storage, Transportation, etc.
G. Waste and Container Cleanup and Disposal

Panel members were John Lyman, Cliff Lehman, Darrell Kenops all of the U. S. Forest Service; Bob Bergland of Washington State Dept. of Natural Resources and Bill Reese, independent thinning contractor. There was considerable floor discussion that is considered in the following summary. The guidelines, for chemical thinning on the Colville and Siuslaw National Forests are in Appendix 8.

A. Selection of Chemical: Only two chemicals were considered, MSMA and cacodylic acid. There was divergence of opinion on silvicultural effectiveness. The Colville Forest strongly favors MSMA because of more complete kill on Douglas-fir and tolerant species; and because translocation to crop trees has been less of a problem. Some other users favored cacodylic acid.

   Formulation: Panel recommends a thickened mixture to reduce drip and splatter problems and formulations that contain a highly visible color. Other additives may be of questionable value.

B. Packaging: Areas of concern were (1) risk in transferring chemical; (2) identification; and (3) accountability.

   (1) Risk of spillage and exposure must and can be minimized by careful transfer procedures between shipping container and application equipment. An alternative is to ship in the container to be used with applicator, thereby reducing the transfer frequency.

   (2) All containers, funnels, etc. must be clearly identified so that other inadvertent uses are minimized, eg. not used or mistaken for fuel, lubricant, kool-aid, etc. There may be applicable state laws and Environmental Protection Agency guides. Users should check.

   (3) Accountability: All containers must be checked out and in for each man each day so that none are left in an unauthorized area.

   Some Chemical companies cooperate in "Return to Distributor" programs for used containers. This might be a valuable service for the user.

C. Application Equipment: "Hack and Squirt" methods using two tools are considered safer. Automatic injection hatchets tend to splatter droplets of the chemical and to drip.

   Tools to make frills for the chemical vary widely. The ideal tool should make a frill or pocket that will not allow the

chemical to runout.
The applicator should accurately dispense the desired quantity of chemical into the frill at very low velocity with no drips. Several satisfactory plastic or corrosion resistant metal products are available. (See Colville and Siuslaw guidelines).

D. Protective Equipment: Several crews have worked for sustained periods without protective equipment other than clean gloves, however, the following protective equipment is recommended.
(1) Plastic or plastic faced gloves - clean and in good condition.
(2) Protective silicon cream on exposed skin areas.
(3) Face shields (probably not useable in all weather conditions - rain, cold, snow). With automatic injection hatchets, face shields seem necessary.

E. Special crew precautions: This seemed to be well covered by Siuslaw and Colville guides and by other panel. Working rules of cleanliness, washing, smoking, eating, etc. need continual emphasis to minimize exposure and spillage.

F. Storage and Transportation: Safety codes for hazardous chemicals and manufacturers recommendations are applicable. Wear and corrosion must be monitored so containers don't leak.

G. Waste and Container Cleanup and Disposal: The Seattle office of Environmental Protection Agency has issued "Interim Recommendations for Excess Pesticide Disposal -- September 1972". These are partially applicable to this problem. County sanitarians should be consulted. State and local codes must be heeded. If not directed otherwise by above codes or guides, burying under at least one foot of mineral soil is probably adequate for minor amounts of waste, wash water, etc. Significant quantities of contaminated vessels, clothing, equipment, water or oil should be disposed in major pits or earth fills which are not near bodies of water. All items disposed of in this way must be immediately covered with one foot of dirt.

MONITORING THE OPERATIONAL LEVEL THINNING PROJECT

Logan A. Norris
Pacific Northwest Forest And Range Experiment Station
Corvallis, Oregon

The purpose of a monitoring program is to determine if sensitive compartments of the environment are receiving an unacceptable level of exposure to the chemical. Sensitive parts of the environment are (1) people, (2) animals, and (3) ground and surface water and vegetation (environmental samples).

Monitoring arsenic in animals and in environmental samples are not required in operational thinning projects. The research base developed in the cooperative arsenic studies indicates arsenic residues are not a significant problem in water, vegetation, or animals when proper chemical application and handling techniques are used. Although human exposure can also be minimized, there are several reasons why monitoring human exposure is necessary.

1. Protect the applicator. Protection of human health is of utmost importance. Estimates of chemical impact on human health require knowledge of exposure level. Arsenic in urine has proven to be a valuable indicator of applicator exposure to silvicide.

2. Protect the employer. The use of a urine monitoring program will provide the employer with a measure of the level of applicator exposure to the chemical. The employer has a responsibility to protect the employee by insuring he does not receive an unacceptable level of exposure to the chemical. The employer can reduce his own liability by insuring applicator exposure is minimized and by having an accurate measure of applicator exposure in the event claims of health damage are filed.

3. Protect the quality of the environment. The results of the 1971 thinning season studies indicate the levels of arsenic in urine are a useful index to the care with which the chemical is handled and applied. Applicators with high levels of arsenic in their urine were associated with elevated levels of arsenic in animals, and therefore, very likely with arsenic residues in vegetation, forest floor and soil.

Results from monitoring arsenic in urine give the field supervisor and professional staff quantitative indicators of the degree to which the crew is adhering to thinning safety guidelines. A combination of quantitative indicators and visual observation can be combined to make a strong case to crew members for adjustment of poor chemical handling and application techniques.

The following urine-monitoring program is suggested for operational level thinning with MSMA and cacodylic acid.

1. Inexperienced crews.
   One pre-exposure urine sample per applicator.
   One urine sample per applicator per week for new crews or new crew members for three weeks (operational stability should be reached in this time).
   One urine sample each three weeks, on the average, for the duration of the exposure period.
2. Experienced crews.
   One urine sample per applicator each three weeks, on the

average, for the duration of the exposure period.

Unexposed forest workers can serve as controls. Collect one urine sample per worker each six weeks for the duration of the thinning program. Four workers will usually be a sufficient number for controls.

All urine samples should be single samples collected near the end of the work day (noon to 7 p.m.), late in a work week (Thursday or Friday). To prevent sample contamination, hands must be washed before collection. Sample container must be protected from any contact with silvicide before and after collection is made.

The sampling schedule is not rigid. Flexibility is encouraged. Careful chemical handling and application techniques are required each day, not just on the day the crew expects to have urine samples collected. Professional staff may want to set urine collection dates on a schedule which is not known in advance by the field supervisor or crew members.

Before a urine monitoring program is initiated, some provision for sample analysis must be made. Details about sample containers, labelling, storage, shipment and return of results must be worked out in detail with the analytical laboratory. It is important that urine samples be shipped for analysis as soon as possible after collection, and that analysis be made promptly when samples arrive at the laboratory. A system of notifying the laboratory when samples are on the way may be helpful. Some provision for reporting high values quickly is essential. Timeliness in the collection and analysis of urine samples and prompt reporting of results are necessary to achieve full value from the urine-arsenic monitoring program.

A technique to permit analysis for arsenic in urine at the Ranger District or Forest Headquarters is under development at Oregon State University. A supplement to this report will be issued when the development and testing phases of the work is completed.

A program to monitor arsenic in urine from applicators should be included in operational thinning projects using MSMA or cacodylic acid. Protection of the employee, the employer, and the quality of the environment are to be gained. The costs incurred are part of doing the whole job and should be included when determining the economic feasibility of chemical versus mechanical thinning.

CONCLUSIONS

APPLICATOR EXPOSURE AND EFFECTS ON HUMAN HEALTH

1. Arsenic in urine is a good indicator to the level of applicator exposure. Arsenic levels rise and fall rapidly with the beginning and end of an exposure period. Average arsenic levels do not appear to increase with duration of exposure. The concentration of arsenic and volume of urine in a single urine sample collected at the end of a work day can be used to give an acceptable approximation of the total amount of arsenic excreted in a 24-hour period.
2. Arsenic levels in urine from applicators sometimes exceed 0.3 ppm which is the level recommended by one manufacturer as an upper safe level. The appropriateness of this standard is questionable and may be considerably below the threshold response level. However, a suitable alternate value has not been found, and most thinning crews are currently operating comfortably within this limit. Five of seven thinning crews working at the operational level in 1971 had less than 5% of Friday urine arsenic concentration observations greater than 0.3 ppm. Intensive training, use of appropriate protective gear, and close supervision will minimize applicator exposure and reduce the maximum urine arsenic levels to a low level (less than 5%, greater than 0.3 ppm). The 0.3 ppm arsenic in urine level should be retained as an indicator of excessive applicator exposure.
3. No apparent significant health problems are associated with the application of organic arsenical herbicides by forest workers when appropriate equipment, protective gear and application techniques, are used.

BEHAVIOR OF ARSENICALS IN THE FOREST

1. MSMA and cacodylic acid are rapidly leached from the surface of the forest floor to the surface of the mineral soil. Little or no leaching occurs in mineral soil. Contamination of ground water by leaching is unlikely.
2. Careful application of MSMA and cacodylic acid cause only slight increases in arsenic levels in browse and grass under treated trees. The concentrations are far below toxic levels, and in nearly all cases, are within FDA tolerances for cacodylic acid in cottonseed hulls for cattle feed. Arsenic levels in forest floor and soil increase after thinning, but in general, increases are usually small. In a ponderosa pine stand which had been scarified and had no pretreatment forest floor material, arsenic levels climbed dramatically 1 year after thinning but declined sharply by the end of the second year. In normal circumstance, "old" forest floor material will dilute fresh fall litter from treated trees. There were little or no changes in soil arsenic levels suggesting the loss of arsenic from the forest floor was not by translocation into the soil profile. The arsenic in MSMA and cacodylic acid can be converted to arsine or arsine derivatives by molds indigenous to the forest. Arsine is gaseous and may be the mechanism by which arsenic leaves treated areas.

3. No significant arsenic residues will occur in streams flowing through chemically thinned areas where the chemical is handled and applied carefully.

ARSENIC RESIDUES AND THEIR IMPACT IN CATTLE, SMALL MAMMALS AND MICROBES

1. No cattle mortality occurred among grazing animals during the 1970 grazing season in areas treated with MSMA or cacodylic acid on operational basis in the 1969 or 1970 grazing seasons. Arsenic residues were not detected in several tissues from two steers and one deer taken from an area thinned operationally during the 1970 grazing season. Cattle can be grazed in areas which are being thinned with MSMA and cacodylic acid if the chemical is being applied by operationally stable crews using prescribed chemical handling and application techniques. Careful supervision is essential to insure cattle are not exposed to the chemical.
2. MSMA is more toxic to cattle than previously believed. Markedly elevated levels of arsenic were in several body tissues from animals fed either cacodylic acid or MSMA. In general, however, levels were lower in animals exposed to cacodylic acid. Diagnostically significant lesions were in kidneys of animals fed cacodylic acid or MSMA. Irreversible renal tubular degeneration was detected. Cacodylic acid is less toxic than MSMA in chronic exposures. The concentrations of arsenic required to cause cattle deaths should not occur in thinned areas when proper chemical handling and application techniques are used.
3. Mice are a sensitive indicator to the use of arsenic for thinning. There is no marked difference in arsenic residues in mice from areas thinned with MSMA or cacodylic acid. Residues are markedly lower and less persistent in mice from western Washington forests than in mice from eastern Washington forests. Arsenic residues in mice appear to reach a maximum within 2 weeks of thinning and then decline with time. Many animals do not contain detectable arsenic residues at any time after thinning.
4. Snowshoe hares are sensitive to MSMA and exhibit an LD_{50} of about 175 mg MSMA per kg body weight. Hares exposed to fairly large single oral doses can be put into two groups based on arsenic residues in their body tissues. Most of the animals than had high arsenic levels died shortly after exposure. Those that survived for several days and were sacrificed, usually contained much lower arsenic residues, indicating rapid excretion of the arsenic. Improper chemical handling and application techniques may result in snowshoe hare deaths. Hares which are alive and show normal levels of activity at capture are probably safe for human consumption. Those wnich are found dead, in distress, or not displaying normal levels of activity may contain high levels of arsenic and are not fit for consumption.
5. The growth and survival, and the carbon and nitrogen metabolism of soil and forest floor micro-organisms were not adversely affected by either MSMA or cacodylic acid at concentrations which are likely to occur in thinned forest stands.

OPERATIONAL LEVEL THINNING OPERATIONS

1. MSMA and cacodylic acid can be applied with an acceptable level of human and animal exposure to arsenic. Careful training, use of protective gear, and good chemical handling and application techniques, and strong, effective field supervision are required.
2. Both the Colville and Siuslaw National Forest guidelines for thinning are acceptable. Some modification of specific items, protective gear, application equipment, or procedures may be required to meet some peculiar situations.

THE KEY TO SAFE USE, HOWEVER, IS TO MINIMIZE EXPOSURE OF THE APPLICATOR AND TO ANIMALS.

APPENDIX

APPENDIX 1

Participating Agencies, Institutions and Cooperators

USDA Forest Service
- Region 1,
  - Colville National Forest and Kaniksu National Forest
- Region 6,
  - Wenatchee National Forest and Siuslaw National Forest
- Pacific Northwest Forest and Range Experiment Station

USDI Bureau of Land Management

Bureau of Sport Fisheries and Wildlife

State of Washington
- Department of Social and Health Sciences
- Department of Natural Resources
- Department of Game
- Washington State University, Department of Veterinary Medicine

State of Oregon,Oregon State University
- School of Forestry
- Environmental Health Sciences Center
- Department of Agricultural Chemistry
- Department of Veterinary Medicine

Dr. Hugh Maycumber,DVM
Key Chemical Company
Ansul Company

APPENDIX 2

Reprinted from the Archives of Environmental Health
April 1972, Volume 24

Arsenic Levels in Urine of Forest Workers Applying Silvicides

Robert F. Tarrant, Corvallis, Ore, and Jack Allard, PhD, Wenatchee, Wash

Forest tree thinning workers absorb arsenic from silvicides. Much of the chemical appears to be excreted from the body in a short time. There appears to be no evidence of a continuing increase in arsenic levels over a period of more than two months. Persons using these materials should be trained to observe all necessary precautions to minimize their exposure.

THINNING overdense stands to improve growth rate of trees is one way in which the nation's supply of merchantable timber can be increased significantly.[1] In the Pacific Northwest alone, several million acres of such dense precommercial stands would benefit from thinning.

Two basic methods are used to thin dense forest stands—felling unwanted trees with saws or killing them with chemicals. Economics aside, thinning by felling concentrates slash on the ground and presents a fire hazard, unsightly appearance, and decreased access to forage for livestock and game animals. Chemically killed trees are left standing. The leaves or needles fall first, then the branches, and eventually the stems. Chemical thinning allows better use of forage, is esthetically more appealing, and presumably is less of a fire hazard. For these and other reasons, the possibility of using chemicals to kill unwanted trees is of great interest.

Cacodylic acid (dimethylarsinic acid) and monosodium methanearsonate (MSMA) are used by public and private forestry agencies in chemical tree thinning programs throughout the Pacific Northwest and elsewhere in the United States. The chemicals are injected into the tree cambium by a variety of techniques, all of which may expose the worker to the chemicals. Since this forest management technique is used widely, the matter of human safety is of concern.

In 1968, the US Forest Service and the Washington State Department of Health, through its Community Pesticide Study project headquartered at Wenatchee, Wash, obtained preliminary data on the level of arsenic in three thinning crew workers exposed to this element. These observations indicated need for a larger, more sensitive study of the potential for arsenic intake by forest workers using arsenical silvicides. In the study reported here, total arsenic concentration, determined by arsenic levels in the urine, was related to method of application, chemical, and length of time of exposure.

Methods

Three six-man crews from the Wenatchee and Okanogan National Forests in north-cen-

Submitted for publication July 1, 1971; accepted Dec 6.

From Forestry Sciences Laboratory, Pacific Northwest Forest and Range Experiment Station, Forest Service, US Department of Agriculture, Corvallis, Ore (Mr. Tarrant), and Community Pesticide Study Project, Washington State Department of Health, Wenatchee, Wash (Dr. Allard).

Reprint requests to Pacific Northwest Forest and Range Experiment Station, PO Box 3141, Portland, Ore 97208.

tral Washington were selected for study. Thinning procedures assigned to each of the six men in each crew were recorded in Table 1.

Crewman 1 was a Forest Service employee living and working in the same area as the thinning crew members but not working with chemicals. Crewmen 2 and 3 used the injection hatchet to apply chemical to trees. This tool automatically meters a prescribed amount of chemical into the tree cambium when the blade strikes the tree. Crewmen 4 and 5 used the hack-squirt method of applying the chemical. This involves making a hatchet cut in the tree, into which the chemical is introduced from a plastic squeeze bottle. Crewman 6 used an injector tool which, after impact with the base of the tree, is manipulated to inject chemical from a reservoir in the handle into the cambium layer.

In order to minimize variations in exposure, all study subjects were required to wear similar clothing and use the same safety practices. Every morning each crew member was supplied a set of freshly laundered outer garments consisting of trousers, a shirt, and two pairs of cotton gloves. The gloves, worn at all times while applying the chemicals, were changed at noon or when they became contaminated. Eye protection consisted of goggles or "wrapa-round" sunglasses. Adequate cleaning facilities were maintained in the field for each man. The study members were asked to wash their hands before smoking or eating. Crew members filled, cleaned, and generally maintained their own equipment. The chemical dispensing apparatuses were dismantled and thoroughly cleaned at least once a day. Major repairs to equipment were made by the crew foreman. Empty containers and wash water were disposed of by the study members under supervision of the foreman.

The study was carried out during the summer months over a period of nine weeks. Urine samples were collected from each worker in each crew on Monday morning before work was begun and again on the following Friday afternoon at the end of the working day.

Urine was analyzed for total arsenic content by digesting a 100-ml aliquot of sample with a nitric-sulfuric acid mixture to oxidize all organic material. The remaining solution was analyzed for total arsenic by converting any arsenates present to arsine gas, which was trapped in a solution containing silver diethyldithiocarbamate. Arsine in the presence of this carbamate forms a colored complex. The color intensity was measured, and the amount of arsenic present in the original urine sample was calculated. In lieu of 24-hour collections, urine concentration differences were corrected by use of osmolality values. The analytical method[2] proved sensitive to 0.01 ppm. Arsenic recovery ranged between 85% and 100%.

Table 1.—Thinning Procedures Assigned to Men in Each Crew

Crewman	Chemical Used	Application Method
1	None	None
2	Cacodylic acid	Injection hatchet
3	MSMA	Injection hatchet
4	Cacodylic acid	Hack-squirt
5	MSMA	Hack-squirt
6	MSMA	Injector tool

Results and Comment

Absence of workers for a variety of reasons made it difficult to obtain urine samples from every study participant at every sampling time. Data are missing in at least one instance for every week except the fourth (Table 2). However, complete data are available for weeks 2, 3, 4, 7, and 8, except for the treatment by the injection hatchet with cacodylic acid. Results of an analysis of variance performed on these complete data indicate that the arsenic content of urine of workers exposed to chemicals was significantly higher than that of workers in the control group (Table 3). There were no significant differences among the noncontrol treatments.

Concentration levels of total arsenic in urine were elevated after a week of exposure to the chemicals regardless of the method of application or chemical used. The high values observed on Friday were, in most instances, near normal again on Monday. In individual cases where a very high value (over 1 ppm) was measured on Friday, the weekend absence from contact with the chemicals did not allow sufficient time for arsenic levels to return to normal. Therefore, Monday values were relatively high in these instances.

Greatly elevated arsenic values could generally be explained as the result of unusual exposure, eg, the men may have accidently cut themselves with hatchets, spilled chemicals on their clothing and skin, had to make frequent equipment repairs, or may have become lax in their safety habits. Statistical analysis shows that arsenic concentrations in urine on Fridays were significantly great-

er than those measured in samples taken on Mondays (Table 3). Thus, we conclude that the generally higher values found on Friday were real and that arsenic concentration in urine will increase during any work week. However, there was no indication of a continuing increase over the nine-week study period.

Complete data were available for all treatments, including that with the injection hatchet with cacodylic acid only, on the basis of Friday observations for weeks 2, 3, 4, and 6. These data were analyzed to compare arsenic levels in urine on the basis of chemical used and on the two methods of application, hack-squirt and injection hatchet. The difference between chemicals was not statistically significant (Table 4). Neither was the difference between application methods, although with both MSMA and cacodylic acid arsenic concentration of urine of workers using the injection hatchet was substantially greater than that of those employing the hack-squirt method.

The arsenic level of urine at which concern for matters of health should begin is not well known. A manufacturer of arsenical silvicides states: Because some individuals are more sensitive than others, it is advisable to always be on the lookout for signs of skin sensitivity among persons handling arsenicals. When a person shows sensitivity, he should be removed from exposure. When an individual is exposed daily for extended periods, the inspection for skin sensitivity should be supplemented by monthly urine analysis for arsenic. . . . When a sample shows above about 0.3 mg of arsenic per liter, another sample should be taken and if again above this figure, the donor should be removed

Table 2.—Total Arsenic Concentration in Urine of Forest Workers Variously Exposed to Arsenical Silvicides: Means of Three Observations

Treatment	Week 1	2	3	4	5	6	7	8	9
	Total Arsenic (ppm)								
Monday									
Control, no exposure	...	0.03	0.05	0.01	0.02	0.03	0.07	0.02	0.07
Injector, MSMA	...	0.08	0.07	0.09	0.05	...	0.10	0.13	0.05
Hack-squirt, MSMA	0.02	0.06	0.04	0.07	0.07	0.10	0.12	0.07	...
Hack-squirt, cacodylic acid	0.02	0.09	0.06	0.06	...	0.15	0.12	0.09	0.09
Injection hatchet, MSMA	...	0.09	0.08	0.04	0.05	0.12	0.13	0.17	...
Injection hatchet, cacodylic acid	...	...	...	0.20	0.21	...	0.37	...	...
Friday									
Control, no exposure	...	0.06	0.04	0.05	...	0.03	0.04	0.14	...
Injector, MSMA	0.18	0.35	0.24	0.58	0.25	0.34	0.56	0.07	...
Hack-squirt, MSMA	0.27	0.26	0.30	0.19	0.23	0.42	0.36	0.20	0.22
Hack-squirt, cacodylic acid	0.22	0.40	0.28	0.51	0.31	0.50	0.51	0.32	0.29
Injection hatchet, MSMA	0.35	0.93	0.26	0.49	0.24	0.54	0.28	0.53	...
Injection hatchet, cacodylic acid	...	0.64	0.76	0.50	0.75	0.88	...	...	...

* Data missing for at least one of three replications (workers).

Table 3.—Means of Three Observations of Arsenic Levels in Exposed Workers for Weeks 2, 3, 4, 7, and 8

Treatment*	Total Arsenic (ppm) Monday	Friday
Control, no exposure	0.04 ± 0.01	0.07 ± 0.03
Injector, MSMA	0.10 ± 0.01	0.36 ± 0.07
Hack-squirt, cacodylic acid	0.03 ± 0.01	0.41 ± 0.08
Hack-squirt, MSMA	0.07 ± 0.01	0.26 ± 0.05
Injection hatchet, MSMA†	0.10 ± 0.01	0.50 ± 0.12
Av of all treatments	0.08 ± 0.01	0.32 ± 0.03

* Control is significantly lower (5% level of probability) than all chemical treatments. No chemical treatment is significantly different from any other. Monday concentrations are significantly lower than those of Friday.

† Injection hatchet-cacodylic acid treatment not included in analysis because of missing data. Means (in ppm) for weeks 4 and 5 are: Monday 0.21; Friday, 0.63.

Table 4.—Mean Total Arsenic Concentration in Exposed Workers by Chemical and Method of Application

Treatment	Mean* ± SE (ppm)
Chemicals	
MSMA	0.42 ± 0.08
Cacodylic acid	0.56 ± 0.09
Application methods	
Hack-squirt	0.36 ± 0.05
Injection hatchet	0.62 ± 0.10

* Differences between the two chemical means and between the two method means were not significant when tested at the 5% probability level.

Table 5.—Comparison of Friday and Following Monday Levels of Arsenic and Observations in Which Total Arsenic Exceeded 0.3 ppm

Treatment	Total Observations	Observations in Which Total Arsenic Exceeded 0.3 ppm	
		Friday	Following Monday
Control, no exposure	23	1	0
Hack-squirt, MSMA	25	8	0
Injector, MSMA	24	10	0
Hack-squirt, cacodylic acid	24	11	0
Injection hatchet, cacodylic acid	18	12	5
Injection hatchet, MSMA	24	13	0

from exposure. Actual experience with . . . (persons involved in the manufacturing operation of arsenical materials) . . . shows that removal from exposure quickly reduces the urine arsenic level to normal and eliminates the signs of skin sensitivity.[3]

All but one of the 15 men applying the chemicals in this study had urine arsenic levels in excess of 0.3 ppm at least once during their period of exposure. The highest level recorded was 2.5 ppm. However, except for those workers using the injection hatchet with cacodylic acid, in all subjects in whom the Friday arsenic level exceeded 0.3 ppm, the level on the following Monday was always less than 0.3 ppm (Table 5).

No health problems were encountered in the study group that could be classified as arsenic poisoning. There were 136 man-weeks of exposure to arsenical silvicides recorded for study members. During that exposure period, 14 men complained of headaches, six of abdominal cramps, five of weakness, four of nausea, and one of skin rash. The controls, observed for 27 man-weeks, had one episode of nausea and one of weakness. The number of persons, especially controls, under observation was small; and no firm conclusions can be drawn, although the large number of reports of headache may have some significance. The crew members were aware that they were part of a health study of an arsenic compound and, as a result, may have overreported symptoms. They did not, however, have an increased rate of sick leave over other Forest Service employees.

Conclusions

Forest tree thinning workers absorb arsenic from silvicides, much of which appears to be excreted from the body in a short time. Measurements over a period of more than two months indicated there was no increase in levels of arsenic in urine after the first week. However, excretion was always greater at the end of each work week than after weekend absence from contact with the chemicals.

Most workers in this study exceeded a total arsenic concentration of 0.3 ppm in urine on one or more occasions during the study. No evaluation of the health effects of this exposure can be made from this study.

The amount of arsenic absorbed by workers could not be related to kinds of arsenical chemicals used or to methods of application.

This study indicates the possibility that tree thinning workers are exposed to excessive quantities of arsenic. Thinning crews using these materials must be trained to avoid skin contact with the chemicals. Protective clothing, including gloves of material impervious to the chemical solution, is most necessary. Water-repellent cream should be used on the face and any other skin areas that cannot otherwise be protected. Extreme care must always be used in handling chemicals, especially when filling application tools where the danger of spillage is great. Furthermore, facilities must be provided for thorough hand and face washing after periods of chemical use.

References

1. *Douglas-Fir Supply Study*. Portland, Ore, US Dept of Agriculture Forest Service, 1969.

2. Steckel LM, Hall JR: *Spectro-Photometric Determination of Trace Arsenic in Aluminum Alloys, Aluminum, Uranium, and Lead*, AEC Research and Development report Y-1406 (chemistry). Atomic Energy Commission, 1962.

3. *Safety and Handling of Ansul Herbicides*, bulletin. Ansul Co Chemical Products Division.

Printed and Published in the United States of America

APPENDIX 3

ARSENIC IN BLOOD AND URINE OF FOREST WORKERS APPLYING DIMETHYLARSENIC ACID

by

Sheldon L. Wagner, M.D.
and
Paul Weswig, Ph.D.

Department of Agricultural Chemistry
and
Environment Health Sciences Center
Oregon State University
Corvallis, Oregon 97331

The recent introduction of organic arsenicals as herbicides in the forestry industry has led to the recognition that neither the safety of these compounds nor threshold limit values of excretion are well established. During the summer of 1971, a five man crew of young healthy white male adults was employed by the Forest Service, U.S.D.A. at Waldport, Oregon. Thsese men worked a five day week thinning young, dense stands of Douglas fir using dimethylarsenic acid (cacodylic acid). Because of the potential human hazards from chronic arsenic exposure, these individuals were studied with the following objectives in mind: a) to determine the amount and ratio of arsenic accumulation in the blood and urine, b) to observe the exposed workers for possible patterns of arsenic intoxication as judged by clinical symptoms, and c) to determine, if possible, threshold limits for excretion of arsenic in urine or levels of arsenic in blood.

METHODS

Five occupationally exposed crewman employed by the Forest Service at Waldport, Oregon were initially interviewed and received a detailed medical history and a physical examination. Particular attention was paid to the past medical history for findings suggesting existing or prior skin, intestinal, neuro-psychiatric, or hematological disease. None of the five members was found to have any significant history or physical findings. These workers were then advised as to safe procedures in the handling of dimethylarsenic acid. They were required to wear protective clothing which consisted of tight trousers, shirts, and workshoes. Intially, they were also supplied with goggles for protection of their eyes. However, these were inconvenient,and the workers became aware of some splash to their faces. Consequently, the goggles were replaced by a plastic face mask. In addition,workers were also supplied with cotton gloves with plastic liners to prevent exposure of the skin and subsequent dermal absorption. Workers were educated to the method of "hack and squirt" application of arsenicals to trees, i.e. placing a cut in the bark and applying a premeasured amount of dimethylarsenic acid.

Twenty four hour urine samples were collected weekly in plastic bottles with each sample collection beginning late Thursday afternoon and terminating at the end of the working day on late Friday afternoon. The workers were then transported to a nearby hospital where blood samples for analysis and aliquots of the 24 hour urine sample were taken. These samples were then sent by bus to our laboratories for arsenic analysis. The method used for arsenic analysis was adapted from the one described by Hundley and Underwood (1). The work time of these exposed workers was followed by the foreman of the crew, in case of absence or illness, etc. These records revealed that the average exposure time to dimethylarsenic acid was only 23.7 hours per 40 hour work weeks. The remainder of the time was used for travel to and from different forest areas. Daily records of the amount of the arsenic compound used were kept and revealed that the average exposure to dimethylarsenic acid (dry weight) was only 817 gm/man/ exposure week.

At the end of the summer, following exposure to the arsenic compound, these workers were again interviewed and examined regarding their medical history.

Five unexposed workers from the Forest Service Ranger Station at Waldport, Oregon were selected as controls for 24 hour urine analyses and blood level analyses of arsenic. Monthly, rather than weekly, samples were deemed adequate. These workers were also examined prior to the start of the study but were not seen in follow-up at the end of the summer.

RESULTS

The results of the weekly blood and urine studies performed on these workers are shown in Tables 1 and 2. The initial dates of 7/9/71 and 7/12/71 (week 1) were taken as controls. The initial exposed work week then began 7/15(week 2) and continued on a 5 day week basis through 8/30/71 (week 8). Initially, there appeared to be a distinct rise in the arsenic levels of the blood. This continued through 8/9 (week 5) at which time a drop was seen in all exposed workers. No change could be determined regarding working conditions with the exception that the area was having a severe heat wave. It is conceivable that considerable amounts of arsenic may have been excreted through the sweat and this may account for these lower values. However, even following the heat wave, continued low values persisted for the remainder of the study.

The results of the weekly urine analysis reveals that there was an almost immediate rise in the 24 hour excretion rates of arsenic (Table 2). This continued on a regular basis through the weeks of continuous exposure (weeks 2 through 8). During the first two days of the ninth week, the men were exposed for a total of only 10 hours to dimethylarsenic acid following which they were changed to a different job occupation. A drop in urinary excretion values was immediately noted by the end of the week and continued during the week of 9/15 (week 10), during which time none of the workers was exposed to arsenic.

Table 1
Blood Arsenic Concentration (ppm) of Exposed and Non-exposed Workers - Summer 1971.

	Weeks										
Subject	1	2	3	4	5	6	7	8	9	10	11
Exposed											
1	.03	.03	.27	.22	.16	.07	.03	.03	.03	.03	.01
2	.03	.27	.20	.19	.08	.11	.06	.03	.03	.01	NA
3	.03	.16	.27	.16	.06	.04	.01	.03	.05	.01	.05
4	.08	.23	.27	.16	.01	.03	.02	.03	.05	.01	.01
5	.07	.16	.27	.24	.01	.03	.02	.05	.05	.01	.01
Unexposed											
6	.03					.13					.02
7	.07					NA					.01
8	.03					.08					.02
9	.07					.01					.03
10	.03					.01					NA

Correlation coefficient 1) total As excreted/24 hr period vs volume of urine = 0.37
2) total As excreted/24 hr period vs ppm As in blood = 0.14

NA indicates no analysis performed.

Table 2
Total Urinary Arsenic Concentration (μg/24 hrs)
of Exposed and Non-exposed Workers - Summer 1971.

Subject	1	2	3	4	5	6	7	8	9	10	11
Exposed											
1	15	36	103	104	98	56	241	519	9	8	
2	77	139	106	118	85	185	72	160	7	110	
3	37	104	104	256	118	98	155	130	33	33	
4	13	60	240	241	195	129	310	130	46	212	
5	47	48	154	140	36	47	65	81	27	27	
Unexposed											
6		43				11					13
7		201				NA					4
8		51				16					14
9		44				20					89
10		24				123					11
Averages (γ/24 hr)											
Exposed	38	77	141	172	106	103	169	204	24	68	
Non-exposed		73				42					26

Throughout the study, cooperation of the workers was excellent in all phases. During the entire period of exposure, none of the individuals lost any time from work. One worker did complain of a two day episode of mild nausea, diarrhea and generalized aches. However he did not seek medical attention for this and took no type of medication or time off.

When the workers were reexamined at the completion of the study, all five stated that they would occasionally note splashing of the dimethylarsenic acid in their faces. The plastic face mask was also uncomfortable and they would occasionally work without it in place. None of the workers reported any unusual odor to the dimethylarsenic acid preparation. It may be of significance, however, that all five workers interviewed after the job was completed noticed a strong odor of garlic characteristic of arsine, present in the forest areas where they had previously thinned. This odor was first detected less than 48 hours following the thinning operation and persisted for at least 3 weeks. Because of possible exposure to arsine gas, and the development of a compensated hemolytic anemia, two of the five exposed workers received blood counts, including reticulocyte counts following their exposures. These studies were found to be normal.

In addition to the total daily arsenic excretions noted in the tables, urine was also analyzed for arsenic based upon part-per-million (ppm) excretion level. At no time during the work exposure did any worker exceed a level of 0.2 ppm arsenic concentration.

The correlation coefficient between total arsenic excreted per 24 hours versus volume of urine was found to be 0.37. The total arsenic excreted per 24 hours versus blood arsenic concentration had a correlation coefficient of 0.14. These data therefore substantiates the impression that total urinary excretion of arsenic is a far more sensitive indicator of exposure than arsenic concentration in blood or random urine samples.

DISCUSSION

The use of organic arsenicals in the forestry industry has raised the question of health and safety factors to the exposed workers. Present threshold limits of urinary arsenic excretion were set at 0.3 ppm by one manufacturing company as early as 1959 and were apparently based upon the recommended values of the Wisconsin State Board of Health plus some additional animal studies performed by the company (2). This would appear to be a very conservative value. Unfortunately there are no values of threshold limits for urinary excretion of organic arsenic which have been set by the United States Committee of Governmental and Industrial Hygienists. Also, in other countries, a widespread divergence of opinion regarding threshold limit values exists (3). A review of the medical literature is not helpful in this respect. Although occupational studies on workmen chemically exposed to arsenic salts have been performed, these compounds, with the exception of the study by the Ansol Company, have been based on workers exposed to inorganic salts. Occupational studies do indicate that the chronically exposed worker to inorganic salts will excrete an average of 70 mcgm per liter without symptoms of arsenic intoxication and that levels as high as 5 mgm per liter may

be reached (4). However, it should be emphasized that these figures reflect exposure to inorganic arsenic and a considerably different toxic effect may theoretically occur with the organic compounds with which this report deals. Literature is primarily directed at the effects of acute arsenical poisoning and the effects of long term accumulation or response of the body to relatively low dosages is not clear. In fact, in reference to the role of low dosage, chronic exposure to arsenic and its relationship to cancer, one can find diametrically opposite opinions in the current medical literature (3,5).

It is apparent from this study that workers exposed to organic arsenicals will show positive evidence of exposure by analysis of the 24 hour urinary excretion during the first week of exposure. As is consistent with many other toxic compounds, the authors feel that this analysis should be performed on a 24 hour urine collection rather than a random sample. This procedure alleviates the problems of urinary concentration as well as the possibility of rapid excretion which may occur from the end of the working day through the following morning at which time a random urine sample might be taken.

The discrepancy between our study, showing no worker exceeding a urinary concentration of 0.3 ppm of arsenic, versus the report of Tarrant and Allard (6) where 14 out of 15 workers exceeded the threshold limit may be at least partially explained upon our differences in collection. However, the biggest factors are probably careful crew supervision, education, and the use of protective gear. Consistent with previous studies, our data do indicate that the compound is readily excreted and that removal from exposure leads to a marked drop in urinary excretion in less than one week.

There was no evidence from our data that whole blood arsenic levels represent a good indicator of arsenic exposure. Although there was a distinct rise which occurred during the first month of exposure, these levels returned to near base line and did not subsequently continue to rise.

During the two months of intensive exposure to dimethylarsenic acid, none of the workers complained of symptoms or had signs suggesting arsenical intoxication. This would indicate that the compound is safe to use when proper safety precautions are taken. Because there was no illness during the study period, we cannot attempt to set threshold limits for excretion based upon these limited studies. Further studies with experimental animals were a clear-cut dose response relationship can be determined may be the technique which will have to be utilized for this type of information. At the present time, it would appear that workers exposed to dimethylarsenic acid should be monitored regularly regarding increased arsenic excretion although the frequency of this determination may not be necessary at weekly intervals. The importance of safeguards such as the proper clothing during application cannot be overemphasized.

The subjective observation of the workers that forestry areas which have been recently treated with dimethylarsenic acid have an extremely strong odor of garlic should not be taken lightly. This may indicate transformation of the compound to arsine gas, documentation of which has already been reported to occur (7). If this is the case, observations of the urinary excretion of arsenic may have little meaning as the toxic properties of arsine gas are much more severe and are markedly different than those of the arsenic salts. In dealing with forestry workers, or other agricultural workers exposed to organic arsenicals, one should also be alert to the development of either central nervous system or hematological toxicity. At this time, a system for monitoring arsine gas in treated areas is also indicated.

ACKNOWLEDGMENT

The authors are grateful for the assistance of Stanley Elliot in the laboratory analysis. Supported in part by the U.S. Public Health Service Research Grant ES-00210 from the National Institute of Environmental Health Sciences and through supplement 90 of the master memorandum of understanding between Oregon State University and the U.S. Forestry Service. Oregon Agricultural Experiment Station, Technical Paper No. 3532.

BIBLIOGRAPHY

1. Hundley, H.K., and Underwood, J.C. Determination of Total Arsenic in Total Diet Samples. JAOAC 53:1176-78 (1970).

2. Safety and Handling of Anusol Herbicides, Bulletin of the Anusol Company, Chemical Products Division.

3. Frost, Douglas, Arsenicals in Biology--Retrospect and Prospect, Federation Proceedings, 26(1):194-208, 1967.

4. Browning, E., Toxicology of Industrial Metals. Butterworth Company, London 1961.

5. Editorial--Arsenic and Cancer, British Medical Journal. 14(2):191. 1968.

6. Tarrant, R.F., and Allard, J., Arsenic Levels in Urine of Forest Workers Applying Silvicides, Archives of Environmental Health, 24:377, 1972.

7. Woolson, E. A. Referred to in "Pesticide Residues: No Danger from Arsenic", Chemical and Engineering News, Page 8, 6 September 1971.

APPENDIX 4

THE ANSUL COMPANY

Marinette, Wisconsin, 54143 Telephone A.C. 715 735-7411
July 25, 1969

Dr. Jack Allard, Director
Community Pesticides Study Program
Washington State Department of Health
P.O. Box 190
Wenatchee, Washington 98801

Dear Dr. Allard:

I have been asked by our sales representative in your area, Robert Lucas, to communicate with you regarding the basis for our use of 0.3 ppm of arsenic in urine as a warning signal for personnel handling cacodylic acid.

We were given a figure of 0.26 ppm by Dr. William Lee of the Wisconsin Department of Public Health about 10 years ago, as a sort of warning level for our Production Department personnel when we first started producing cacodylic acid and disodium methanearsonate (DSMA). At the time the Wisconsin Department of Public Health was analysing urine samples from our personnel on almost a monthly basis. This was for the purpose of gaining experience on the handling of arsenicals. We had a few high levels to begin with, but after tightening upon our handling techniques we ended up with so few cases over 0.26 ppm that after 2 or 3 years we abandoned the urine sampling and analysis.

In preparing our Toxicity and Handling Bulletin on arsenicals several years ago, we simply rounded off the 0.26 ppm figure to 0.3 ppm. This is the origin for this figure.

I do not know where Dr. Lee obtained the 0.26 ppm figure. I do know that he had analysed urine samples for other individuals in Wisconsin, prior to the analysis of samples from Ansul personnel and therefore felt that this was a realistic back-ground level for individuals in this area. Perhaps in areas where a lot of seafood is consumed, this level should be higher.

For futher information on this subject, please refer to the following references:

1. Urinary Excretion of Arsenic, by M.R. Mattice and D. Weisman, Am J. M. Sc. 193, 413-420 (1937).

2. Distribution of Arsenic in Nonexposed Persons, By G.W. Boylen, Jr. and H. L. Hardy, Amer. Ind. Hyge. Assoc. J., March-April 143-150 (1967).

The first article above gives the urinary excretion of arsenic for 163 normal individuals as varying from none to 0.85 mg per day, with an average of 0.26 mg. Assuming a 3 liter urine volume per day the maximum is equivalent to about 0.3 ppm and the average about 0.1 ppm.

I am enclosing a copy of 90 day feeding studies with cacodylic acid on rats and dogs, and a copy of a 10 week feeding study on chickens. In the 90 day studies, there were no effects observable at the maximum levels used, which was 100 ppm (as As) for rats and 30 ppm (as As) for dogs. These levels are equivalent to about 600 mg of arsenic per day for a 70 kg man based on the rat level, and about 50 mg per day based on the dog level. The report of Dr. S. A. Peoples, "The Metabolic Fate of Cacodylic Acid in the Dairy Cow", sent you on November 1, 1967, shows that excretion of cacodylic acid in the cow is mostly in the urine, and reaches a balance between intake and excretion in about 30 days. Assuming 75% of the cacodylic acid entering a 70 kg man is excreted in the urine (the remainder in feces), 50 mg of As per day, based on dogs, should give a urine level in man of about 12 ppm. Allowing a safety factor of 10 should give a level of about of about 1.2 ppm As as a safe upper limit. If there is evidence of skin sensitivity for any one individual, this safe upper limit should not apply to him.

This is about the best advice I can give on raising the warning level arsenic in urine in individuals handling cacodylic acid above the 0.3 ppm level we have used in the past. If you have any questions, please do not hesitate to contact me.

Very truly yours,

THE ANSUL COMPANY

P. J. Ehman
Manager, Special Projects

PJE
mes

Attachment

APPENDIX 5

THE EFFECTS OF LONG TERM FEEDING OF MONOSODIUM ACID METHANEARSENATE (MSMA) TO RABBITS[1,2]

by J. H. Exon, J. R. Harr[3] (Department of Veterinary Medicine)
and R. R. Claeys (Department of Agricultural Chemistry)
Oregon State University, Corvallis, Oregon

ABSTRACT

Adult rabbits were fed 50 ppm MSMA in the feed for 2 to 52 weeks. Toxic hepatitis coincided with daily oral doses of 1.5 mg As/Kg body weight after 7 and 12 weeks exposure. These lesions were not present after the daily oral dose was reduced to .74 mg As/Kg body weight even though the concentration of arsenic in liver tissues increased. Toxicity was related to the oral dose of arsenic and not the resulting tissue concentrations. MSMA resembles more toxic arsenic compounds by a tendency to accumulate in liver and kidney tissue, a high proportion and concentration of arsenic in the feces and evidence of toxic hepatitis produced at relatively low oral doses. Analysis of stomach contents and tissues of offspring of MSMA-fed females indicate arsenic metabolites of this compound do not cross the placenta nor are excreted in the milk.

INTRODUCTION

Arsenic occurs naturally in trivalent and pentavalent, organic and inorganic compounds. Generally, trivalent inorganic forms are more toxic due to inactivation of enzyme systems by covalent bonding to the attached thiol groups (1,2,3,4) resulting in slower excretion rates and accumulation in body tissue. Pentavalent organic forms do not inhibit enzymatic processes (1,2,3,5,6,7), are excreted more rapidly and do not accumulate in body tissues. However, generalizations concerning arsenicals are risky and notable exceptions exist in most cases.

Few long-term feeding studies have reported effects of low doses of arsenicals. This is especially true of the relatively recently discovered pentavalent organic arsenicals used as herbicides and silvicides. MSMA, one of these pentavalent organic arsenicals, is an effective silvicide for pre-commercial thinning of overstocked stands of conifers, but questions concerning the safety of this practice have been raised (8,9).

---

[1]Supported by Pacific Northwest Forest and Range Expt. Stat., U.S. Forest Service, through supplement 70 to the Master Memorandum of Understanding with Oregon State University.
[2]Oregon Agricultural Experiment Station; Technical Paper No. .
[3]New address is; Pennwalt Corporation, Head of Toxicology Division, Rochester, New York 14603.

A total dose of 100 mg MSMA per kilogram body weight, administered at 10 mg MSMA/kilogram body weight/day (5.1 mg As) was lethal to cattle (10). The acute LD_{50} to snowshoe hares was estimated to be 173 mg MSMA/kilogram body weight (88 mg As) (11). Ninety-day feeding studies of cacodylic acid, a related compound, resulted in no adverse effects at doses of 30 ppm (as As) in dogs or 100 ppm (as As) in rats (12).

In this study low levels of MSMA were fed to adult rabbits for one year. Organ and tissue concentrations of arsenic and histopathologic effects are reported herein.

METHODS AND PROCEDURES

Fifty-two six-month old New Zealand White rabbits were divided into three groups according to different exposure regimens. Group I (16 males and 16 females) was fed 50 ppm MSMA (27.5 ppm As) added to a commercial rabbit feed (Table I) and two animals of each sex were necropsied after 2, 4, 7, 12, 17, 24, 36 and 52 weeks of exposure. Group II (four males and four females) was fed the MSMA diet for 12 weeks and then fed the control diet for an additional 5 or 13 weeks at which time two males and two females were necropsied. Group III (eight males and eight females) served as non-exposed controls and were fed the basal commercial ration (Table I). One animal of each sex was necropsied at the same time as those in Group I.

TABLE I

Basal Commercial Ration[1,2]

Alfalfa	45.5%
Barley	40.0%
Soybean Meal	12.0%
Molasses	2.0%
Salt	0.5%

[1]Crude protein 16.5%, digestible protein 12.0%
[2]50 ppm MSMA added to experimental feed at time of manufacture. Both experimental and control feeds were pelleted.

Females from Group I were bred at 9 months of age (12 weeks exposure) and a pair offspring were killed at one and 20 days of age. Samples of liver, kidney and stomach contents were collected from one male and one female of each litter at each age and analyzed for arsenic residues to determine the possibility of arsenic contamination occurring *in uteri* or from nursing females exposed to MSMA.

All rabbits were housed in open rabbit cages and fed ad libitum for the first 10 weeks of exposure. After 10 weeks the ration was cut to 140 g/rabbit/day, as recommended by commercial rabbitries to prevent females from becoming too obese to breed. Feed intake was monitored daily and the rabbits were weighed each week.

At necropsy, sections of liver, kidney, muscle, hair and bone were collected for histologic studies or arsenic residue analysis. Tissues for histological examination were fixed in 10% formalin; those for arsenic residue analysis were frozen. Urine and feces were collected from each rabbit during the 24 hour period prior to necropsy. Concentrations of arsenic in tissue samples were determined by the method of Hundley et al. (13). Tissue samples from individual rabbits were pooled according to age and exposure regimen for analysis of arsenic residues. Pools were composed of tissues from 2 to 4 rabbits, depending on the exposure regimen. Histologic samples of liver, kidney and muscle were sectioned at six microns and stained by the hematoxylin-eosin method (14).

RESULTS

The concentration of arsenic increased rapidly in liver and kidney tissues of Group I rabbits during the first 2 weeks of exposure and then plateaued until 17 weeks when arsenic residues again were increased (Table II). Arsenic residues in hair accumulated slowly during the first

TABLE II

Concentration of Arsenic (ppm) in Tissues and Excrements of Rabbits Exposed to 50 ppm MSMA in the Feed[1]

	Weeks of Exposure				
	2	4	7	12	17
Liver	.57	.37	.44	.37	.78(.17)[2]
Kidney	.56	.47	.56	.49	1.40
Muscle	<.10[3]	.27	.26	.54	.36
Bone	<.10[3]	<.15[3]	<.10[3]	<.10[3]	3.52
Hair	<.25[3]	1.30	2.42	3.58	2.29(.90)[2]
Feces	11.30	8.10	8.50	8.80	8.40
Urine	15.10	9.10	13.30	8.40	6.20(<.10)[2,3]

[1]Values represent analysis of pooled tissue of 2-6 rabbits, depending on the exposure regimen

[2]Values in parenthesis are the arsenic concentration from animals fed 50 ppm MSMA in the feed for 12 weeks and then fed the control ration for 5 weeks.

[3]<=Below reliable limits of detection (for hair=.6 ppm; for other tissue=.15ppm).

12 weeks exposure to MSMA and then plateaued. Bone did not accumulate detectable amounts of arsenic until 17 weeks of exposure. Bone and hair accumulated greater concentrations of arsenic than liver, kidney or muscle. The concentration of arsenic in excretory products of rabbits exposed to MSMA in the feed was highest after 2 weeks of exposure. From 4 to 17 weeks of exposure arsenic residues in the feces remained constant. Urinary arsenic concentrations were more variable but indicated a general tendenty to decrease from 2 to 17 weeks on MSMA feed (Figure 1). Approximately 70% of the ingested arsenic was excreted and of this amount 54% was in the urine and 46% in the feces (Figure 1). The average ratio of arsenic concentration in urine to that in the feces was 1.41.

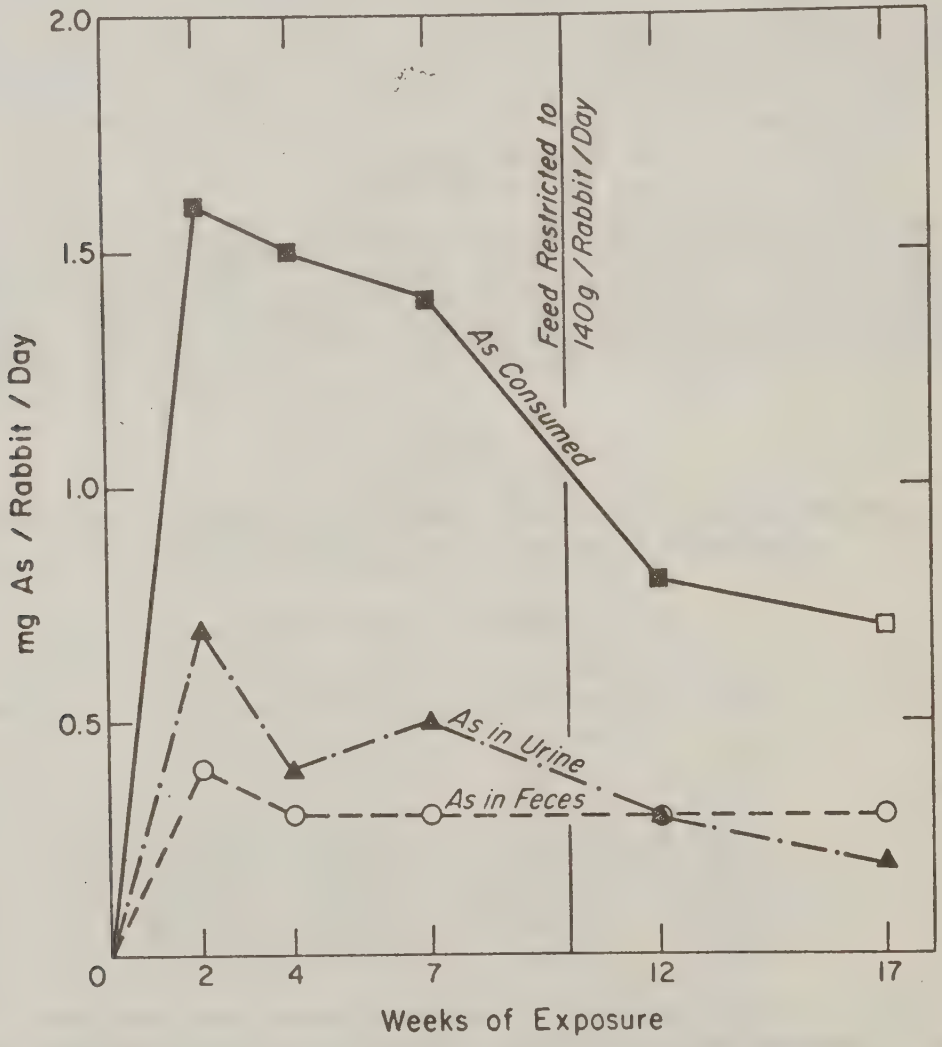


FIGURE 1. Arsenic ingestion and excretion per kilogram body weight by rabbits fed 50 ppm MSMA.

Samples of a soft tissue (liver), a hard tissue (hair) and an excretory product (urine) from Group II animals analyzed 5 weeks after they were taken off the MSMA feed and given control rations indicate arsenic residues had decreased to control levels. The concentraion of arsenic in tissues of control rabbits (Group III) of similar ages was .20, .70, <.15 ppm respectively for liver, hair and urine. Tissues from all animals necropsied at 24, 36 and 52 weeks of exposure were accidently discarded prior to determination of arsenic residues. No detectable arsenic residues were found by analysis of stomach contents, liver or kidney of one or 20 day old offspring of Group I females.

Histologic changes in kidney, muscle and liver tissue of Group I and II rabbits were not different from the controls at comparable age periods, except for the case of liver tissue of 8 rabbits necropsied from Group I at 7 and 12 weeks exposure to MSMA. Toxic hepatitis was present in all eight of these rabbits. In addition reactive hyperplasia was present in two of the four rabbits exposed for 7 weeks and three of the four exposed for 12 weeks. These lesions were not metaplastic or anaplastic. Liver sections from all other rabbits did not contain lesions more severe than vacuolar degeneration.

The disappearance of toxic hepatitis coincided with the lowest rate of arsenic ingestion per kilogram of body weight and the greatest arsenic concentration in liver tissue (Table II).

DISCUSSION

The initial (up to 2 weeks exposure) and latent (after 12 weeks exposure) accumulation of arsenic in liver and kidney tissue, occurrence of primary toxic hepatitis at a relatively low oral dosage rate and the presence of 30% or more of the ingested arsenic in the feces indicate MSMA may be more toxic than most pentavalent organic arsenicals. A recent study of chronic and acute toxicity of MSMA in cattle supports the greater-than-expected toxicity of this compound. Three steers were fed a total dose of 100 mg MSMA/kilogram body weight over a 10 day period, resulting in the death of two and illness of the third (10). This experiment was repeated with a more purified form of MSMA with similar results (10).

It is generally accepted (2,3,5) that organic pentavalent arsenicals are relatively non-toxic compounds which are not accumulated in tissues and are rapidly excreted via the urine. Trivalent inorganic arsenicals are considered more toxic than pentavalent forms, accumulate in tissues and are excreted mainly in the feces (2,3,5). However, reports of the metabolic fate of the pentavalent compounds are conflicting. Frost et al. (5) and others (2,3) deny that pentavalent arsenicals are reduced to the trivalent state *in vivo* while others (2,3,4) postulate that toxic effects of some pentavalent forms are due to an *in vivo* reduction producing the more toxic trivalent compounds.

Previous studies have reported that the affinity of an arsenical to body tissue is directly related to its toxicity regardless of the dose (2,3). However, toxic hepatitis observed in this study was inversely related to the concentration or arsenic in the liver and directly related to the rate of MSMA consumption. Daily oral doses of 1.5 mg As/kilogram body weight for 10 weeks coincided with primary toxic hepatitis. Hepatitis was not observed (assuming the four rabbits necropsied at 12 weeks exposure did not have time to recover) in rabbits autopsied after the daily oral dose was reduced to .74 mg As/ kilogram body weight by restricting the food intake for breeding purposes. Arsenic residues in the liver, following the initial increase in the first 2 weeks of exposure, remained relatively constant until after 12 weeks of exposure. Residues then increased two-fold during the remainder of the experiment while hepatitis was not present.

It appears that the toxicity of MSMA in rabbits is more dependent on the quantity of the oral dosage received during a given time period than to the resulting concentration of arsenic in the tissue. Similar results were obtained with rabbits given p-COOH-phenylarsenoxide by Hogan et al. (3). It is postulated that these compounds may be converted in vivo to a more toxic form. Initiation of this conversion reaction may be dose-related. A dose-related or threshold alteration in the metabolism of some pentavalent organic arsenicals to produce a more toxic metabolite may partially explain conflicting reports of in vivo reduction of these compounds.

The plateauing effect of arsenic residues in the liver from 2 to 12 weeks of exposure may have resulted from the initial stages of hepatitis partially blocking functions necessary for the accumulation of arsenic in the liver. When the amount of arsenic ingested was reduced and hepatitis resolved, arsenic again accumulated in the liver.

Analysis of arsenic residues of one and 20 day old offspring of females fed 50 ppm MSMA for 12-24 weeks indicated that arsenic contamination does not occurr either in uteri or from nursing.

CONCLUSIONS

Monosodium acid methanearsenate resembles the more toxic arsenic compounds by a tendency to accumulate in liver and kidney tissue at initial and terminal exposure periods, a high proportion and concentration of ingested arsenic in the feces and primary toxic hepatitis at a relatively low oral dose. A high rate of arsenic excretion versus ingestion, major use of urinary excretion of arsenic and non-accumulation in muscle tissue resembles the less toxic arsenicals. Properties that are intermediate of more or less toxic arsenicals include the plateauing effect of arsenic residues in liver, kidney, hair and bone after intermediate exposure and the presence of both fecal and urinary excretion of arsenic. These observations indicate that MSMA may be converted to a more toxic state in vivo. It is postulated that this

conversion reaction may be related to increased MSMA consumption which causes the activation of alternate pathways of MSMA metabolism which produce more toxic arsenic metabolites.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Jack Lamberton for his capable technical assistance in the analysis of arsenic residues for this study.

BIBLIOGRAPHY

1. Johnstone, R. M. Sulfhydryl Agents. Metabolic Inhibitors Vol. II, (R. M. Hochester and J. H. Questel, editors), Academic Press, Inc., New York 1963.

2. Schroeder, H. A. and Balassa, J. J. Abnormal Trace Elements in Man: Arsenic. Journal of Chronic Disease 19, 85-106 (1966).

3. Hogan, R. B. and Eagle, H. The Pharmacologic Basis for the Widely Varying Toxicity of Arsenicals. Journal of Pharmacology and Experimental Therapy 80, 93-113 (1944).

4. Sollmann, T. Manual of Pharmacology, 7th Edition. Saunders, Philadelphia 1953.

5. Frost, D. V. Arsenicals in Biology: Retrospect and Prospect. Federal Proceedings 26, 194-208 (1967).

6. Frost, D. V., Overby, L. R. and Spruth, H. C. Studies with Arsanilic Acid and Related Compounds. Journal of Agricultural Food Chemistry 3, 235-243 (1955).

7. Schriber, M. and Brouwer, E. A. Metabolism and Toxicity of Arsenicals. I. Excretion and Distribution Patterns in Rats. Federal Proceedings 23, 199 (1964).

8. Norris, L. A. Studies of the Safety of Organic Arsenical Herbicides as Precommercial Thinning Agents. Precommercial Thinning of Coastal and Intermountain Forests in the Pacific Northwest. Washington State University, Pullman, Washington, 63-74 (1971).

9. Tarrant, R. F. and Allard, J. Arsenic Levels in Urine of Forest Workers Applying Silvicides. Arch. Environ. Health, 24, 277-280 (1972).

10. Dickinson, J. O. Toxicity of the Arsenical Herbicide Monosodium Acid Methanearsenate in Cattle. American Journal of Veterinary Research 33, 9 (1971).

11. Unpublished data from Evans, cited by Norris (1971).

12. Unpublished report of the Wisconsin Alumni Research Foundation.

13. Hundley, H. K. and Underwood, J. C. Determination of Total Arsenic in Diet Samples. Analytical Chemistry 53:6, 1176-1178 (1970).

14. Galigher, A. E. and Kozloff, E. N. Essentials of Practical Microtechnique. Lea and Febiger, Philadelphia (1964) 448 pages.

Toxicity of the Arsenical Herbicide Monosodium Acid Methanearsonate in Cattle

John O. Dickinson, D.V.M., Ph.D.

SUMMARY

The study was conducted to determine the toxicity of monosodium acid methanearsonate (MSMA) in cattle. A total of 5 cattle was treated with solutions of MSMA. Of the 5 cattle, 4 succumbed to the toxic effects of the compound on the renal tubules after they were given a total dosage of 100 mg. of MSMA per kilogram of body weight (10 mg./kg./day for 10 days) or less. Arsenic residues as high as 64 p.p.m. were found in kidney with a minimal content in liver of 27 p.p.m. Toxic nephrosis and hemorrhagic gastritis were seen in all cattle; lesions in liver were variable and were absent from other tissues studied.

Monosodium acid methanearsonate, a widely used organic herbicide, is sometimes used in precommercial thinning of forests. Since cattle often were grazed in areas where MSMA was used, the question of toxicity of the agent to cattle arose. The median lethal dose (LD_{50}) in cattle is not known, but according to one report,[a] repeated doses of 10 mg./kg. produced poisoning in cattle. In this instance, poisoning was considered to have occurred when any sign of abnormal function or behavior was observed.

The oral LD_{50} in mice and in rats are 1,800 and 700 mg./kg., respectively; 120 ml. of MSMA solution is considered sufficient to kill a 68-kg. man.[4]

Monosodium acid methanearsonate contains arsenic in the pentavalent form. Pentavalent organic arsenic is considered less toxic than is the trivalent inorganic form; apparently, the former is less binding to sulfhydryl groups.[1] The pentavalent form, however, may be converted to the trivalent form in the animal body. There is almost no information on the breakdown of MSMA in animal tissues. The fate is presumed to be similar to that of cacodylic acid; namely, unchanged in urinary excretion.

Materials and Methods

Two preparations of MSMA were used: one contained 44.9% MSMA in an aqueous solution plus other ingredients, primarily a dye,[b] and the other contained only 59% MSMA in an aqueous solution.[c] In both preparations, the total arsenic content was 21%. These were administered orally to cattle, using gelatin capsules. A total of 7 yearling white-faced cattle were used; 2 were used as controls (1 steer and 1 heifer), 3 were treated with the dye-containing solution (2 steers and 1 heifer), and 2 were treated with the aqueous solution (1 steer and 1 heifer). Weights ranged from 118 to 217 kg. at the start of the project (Table 1).

A rather small dose was initially given to

Received for publication Nov. 12, 1971.

From the Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine, Washington State University, Pullman, Wash. 99163, where Dr. Dickinson is associate professor.

Supported by U.S. Forest Service Grant No. FS-PNW-1603.

[a] Palmer, J. S., College of Veterinary Medicine, Texas A&M University, College Station, Texas: Personal communication, March 20, 1970.

[b] Glowon Tree Killer, Key Chemicals, Inc., Anacortes, Wash.

[c] Ansar 170 HC, The Ansul Company, Marinette, Wis.

gain information on chronic toxicity of the herbicide. Cattle were treated each day at the dosage level of 10 mg. of MSMA per kilogram of body weight per day. The dosage level of 10 mg./kg./day was continued for 3 weeks; at the end of this period, a hepatic biopsy sample was obtained for arsenic determination. During the 2nd 3-week period, the dosage was doubled to 20 mg./kg./day, and biopsy of liver was done at conclusion of this segment. The 3rd 3-week period completed the study of chronic toxicity; the dosage was increased to 30 mg./kg./day during the final period.

TABLE 1—Test Procedure

Cattle							
No.	Age (mo.)	Sex	Body weight (kg.)	MSMA formulation	Daily dose (mg./kg.)	Duration of treatment (days)	Disposition
3	13	Steer	216	44.9% with dye	10	10	Died, day 10
4	13	Heifer	167	44.9% with dye	10	10	Recovered
5	12	Steer	177	44.9% with dye	10	10	Killed, day 12
12	14	Steer	211	59.0% aqueous	10	8	Died, day 8
15	9	Heifer	118	59.0% aqueous	10	8	Died, day 16
126	14	Steer	209	.... (control)	....	....	Killed, day 12
124	15	Steer	217	.... (control)	....	....	Killed, day 10

At the conclusion of the 9-week experiment, remaining cattle were killed and necropsied, and tissue sections from liver, kidney, brain, and abomasum were examined for gross and microscopic lesions of significance. Hair and tissue samples from all cattle were assayed for arsenic content at the end of the treatment period; hair samples were also assayed at the start of the treatment period. Duplicate analyses were done on all samples, using neutron activation analysis[3,d] and a spectrophotometric method.[2,e]

Results

The procedure as outlined was not completed because the cattle were unable to tolerate the MSMA solution (preparation 1) at the lowest dosage rate for more than 10 days. At this point, 1 steer (No. 3) died, and treatment was stopped on the other 2 (1 steer and 1 heifer). All 3 cattle had developed severe diarrhea by the 7th day of treatment, along with dehydration and body weight loss; these were the first clinical signs of poisoning. Steer 5 became more lethargic and was unable to rise by day 12; this steer was killed (overdose of sodium pentobarbital). Heifer 4 had signs of recovery by this time and was observed each day after treatment was ended. The heifer apparently recovered.

Evidence of gross pathologic changes were seen in kidney and abomasum of steers 3 and 5. Kidneys were markedly swollen. On cut surface of the kidney, medulla appeared darkened, even hemorrhagic, whereas little change was seen in the cortex (Fig. 1). There was hemorrhagic gastritis characterized by mucosal erosion and ulceration and intense hyperemia. A large amount of blood was admixed with abomasal contents.

Results of microscopic examination of abomasum from steers 3 and 5 indicated marked hemorrhagic gastritis. Multiple foci of coagulative necrosis having a midzonal distribution were in the liver of

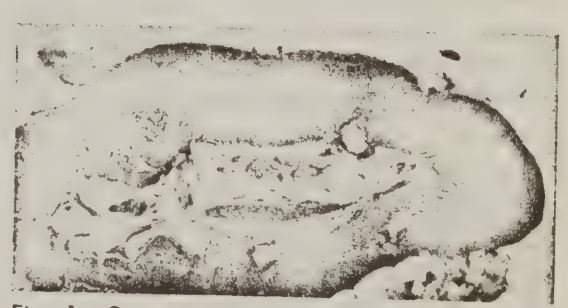


Fig. 1—Cut surface of kidney of steer 3 which was given 10 mg. of monosodium acid methanearsonate per kilogram per day and died on day 10. Notice hemorrhage in the cortex.

[d] Nuclear Radiation Center, Washington State University, Pullman, Wash.

[e] Washington State Division of Health, Wenatchee, Wash.

TABLE 2—Tissue Arsenic Residues (p.p.m.) in Cattle Given Monosodium Acid Methanearsonate (44.9% Solution with Dye)

Tissue*	Steer No. 3			Steer No. 5			Control (steer 126)
	Before treatment	Neutron activation analysis	Spectrophotometric analysis	Before treatment	Neutron activation analysis	Spectrophotometric analysis	Neutron activation analysis
Kidney cortex	N.D.	57.9	64.2	N.D.	26.7	23.2	0.25
Liver	N.D.	27.0	24.9	N.D.	27.4	30.3	0.82
Brain	N.D.	1.8	1.7	N.D.	2.6	1.7	0.05
Bone (rib)	N.D.	4.9	N.D.	N.D.	2.5	N.D.	0.03
Muscle (intercostal)	N.D.	10.3	N.D.	N.D.	7.4	N.D.	0.02
Abomasum (mucosa)	N.D.	N.D.	N.D.	N.D.	14.2	17.7	0.02
Hair (top of back)	2.7	3.3	N.D.	1.1	1.4	N.D.	0.81

* All tissue samples were obtained at necropsy following treatment period, except for hair samples.
N.D. = not done.

steer 3. In steer 5, lesions were not seen in liver or brain. Both steers, however, had marked diffuse nephrosis. It was concluded that renal tubular degeneration was the cause of death of steer 3 and ultimately would have been the cause of death of steer 5.

Tissue arsenic concentrations are recorded (Table 2) for steers 3 and 5; results from both the neutron activation analysis and spectrophotometric method are given. Agreement between the 2 methods was quite satisfactory. Sample of liver was withdrawn from heifer 4 on May 10; the hepatic arsenic content was 7.2 p.p.m.

In steer 12 and heifer 15, given preparation 2 (aqueous solution of 50% MSMA) at the dosage rate of 10 mg. of MSMA per kilogram of body weight per day, diarrhea was evident by day 5. This diarrhea represented the first clinical sign of toxicity. Steer 12 died on the 8th day of treatment. Heifer 15 survived until day 16, even though treatment was discontinued on the 8th day. Gross and microscopic pathologic changes were quite similar to those described for steers 3 and 5. The tissue arsenic concentrations for liver and kidney are given (Table 3).

Discussion

Of the 3 cattle in the 1st group given MSMA (10 mg./kg./day), 2 died from apparent kidney failure—1 steer (No. 3) after 10 days of treatment and the 2nd steer (No. 5) 2 days later. The latter steer was not given MSMA on days 11 or 12, but kidney damage was apparently irreversible.

The hepatic arsenic concentrations for both steers were similar (27 p.p.m.); however, the changes in renal arsenic concentrations were different. After treatment was discontinued in steer 5, the renal arsenic concentration decreased to a value less than the concentration in the liver. It is assumed that the steer was able to remove arsenic from the kidney rather rapidly. Nevertheless, toxic nephrosis was the apparent cause of death. The phenomenon could have diagnostic significance, since organic pentavalent arsenicals are assumed to produce higher renal tissue concentrations than hepatic tissue concentrations in poisonings. Cattle could conceivably deplete the arsenic content in the kidney rather rapidly to a value below that of the liver and perhaps below the value having diagnostic significance, but succumb to irreversible tubular damage.

TABLE 3—Tissue Residues (p.p.m.) in Cattle Given Monosodium Acid Methanearsonate (59% Aqueous Solution)

Tissue*	Spectrophotometric assay		
	Steer 12	Heifer 15	Steer 124
Liver (necropsy sample)	17.7	1.6	0.7
Kidney (necropsy sample)	45.8	3.5	1.1

* All tissue samples were obtained at necropsy following treatment period.

The results obtained by using an aqueous solution of MSMA (preparation 2) were essentially the same as those obtained with the commercial dye-containing product (preparation 1). Toleration of the 10-mg./kg./day dosage was quite similar.

A total dose of 80 to 100 mg./kg. was fatal to 4 of 5 cattle used in the present experiment. Clinical signs of poisoning (diarrhea and anorexia) were evident in all 5 cattle when a total dose of 50 to 70 mg./kg. had been given.

References

1. Goodman, L. S., and Gilman, A.: The Pharmacological Basis of Therapeutics. 2nd ed. The Macmillan Company, New York, N.Y. (1956): 948–949.
2. Steckel, L. M., and Hall, J. R.: A Spectrophotometric Method for Arsenic Determination Using Silver Diethyldithiocarbamate. U.S. Atomic Energy Commission Report Y-1406, 1962.
3. Thomas, J., and Kristensen, L. V.: Determination of Arsenic in Biological Material by Radioactivation Analysis. Acta med. scand. Suppl., 496, (1968): 23–26.
4. Vallee, B. L., Ulmer, D. D., and Wacker, W. E. C.: Arsenic Toxicology and Biochemistry. A.M.A. Arch. Indust. Health, 21, (1960): 132–139.

APPENDIX 7

DAILY DIARY - CREW CONTACT WITH SILVISAR 510

6/23/71 A. Wallat did not work
B. No observed or reported accidents by remainder of the crew.

6/24/71 A. Wallat spilled a quantity of chemical on his leg due to a rusted hole in applicator.

6/25/71 A. Rainy Day
B. Thinned two hours. No reported or observed accidents

6/28/71 A. No reported or observed accidents

6/29/71 A. No reported or observed accidents.

6/30/71 A. McCrum reported two occasions when chemical splashed on his face while applying to a tree.
B. No reported or observed accidents by the remainder of the crew.

7/1/71 A. McCrum reported chemical spilled on leg when chemical container leaked.
B. No reported or observed accidents by the remainder of the crew.

7/2/71 A. No reported or observed accidents.

7/6/71 A. McCrum - in the A.M. reported rash on face believed to be caused by chemical or insect repellent (Cutter). Rash reported to itch badly.
B. No reported or observed accidents by remainder of crew.

7/7/71 Report from Wenatchee revealed chemical level in system of each crew member but McCrum who reported late.

Wallat - 0.20
Melvin - 0.20
Leckner - 0.31)
Booth - 0.63) Above critical point, listed as 0.30
Aman - 0.65)

Held safety meeting and reviewed Forest Service memo on thinning guidelines. We determined that we have two major problems:

1. Equipment - A. We have not been using plastic gloves and have none in supply at the present. Our cotten gloves have accumulated quantities of silvisar on them.
B. Applicators - We have been using metallic oil cans as applicators rather than the recommended plastic. (Both gloves and applicators are on order).

2. Handling Procedure - We determined that we may not have been following points 10, 14, 16, 17 and 18 as closely as we could*. Adjustments were also made in the system of transporting and storing materials which include and come in contact with the chemical and those which do not and should not.

*Thinning Guidelines.

Crew members did not show a great deal of concern when told the situation. They did think that crews should know more about the chemical and its effects. Questions asked included:

1. Which test supplied the figures reported?
2. How serious was their particular situation?
3. What about our crews level as compared to other crews doing the same work? (Colville).
4. Is there a chance of the level decreasing by more careful handling and better equipment?

McCrum's rash did not appear to be much improved.

7/8/71
A. McCrum - rash on face did not appear to have improved. He was driven to Newport, Washington, to see a doctor.
B. Leckner reported that he got one glove wet with chemical during day.
C. Wallat noticed a few splashes on his shirt and face at noon.
D. No reported or observed accidents by remainder of crew.
E. McCrum - Doctor prescribed ointment for use on rash. Reported him okay for return to work.

7/9/71
A. McCrum - back on job.
B. Received shipment of plastic gloves and began using them.
C. Received second report from Wenatchee on chemical level in system. All reported below significant except Wallat (0.33).

7/12/71
A. No reported or observed accidents.
B. McCrum reported he had Saturday appointment with family doctor concerning rash on face. Will report his doctor's conclusions.

7/13/71
A. No reported or observed accidents.

7/14/71
A. No reported or observed accidents.

7/15/71
A. McCrum - Spilled a quantity of chemical on shirt from gallon container.

7/16/71
A. Did not thin - were sent on mop-up detail on small fire.

7/19/71
A. Did not thin.
B. Brushed road most of day.
C. About 4:00 P.M. pumped Silvisar 510 into empty 5 gallon containers. Aman splashed some Silvisar into open blister. He washed the blister out and his hands immediately.

7/20/71 A. New plastic applicators arrived and we put them to use.
B. Complaints that the chemical tends to drip out the nozzle after application has been completed.
C. Plastic gloves tear easily in brush. Large hands tend to make them tear between fingers and at finger tips.
D. Have a difficult time making gloves last half a week.
E. Held morning meeting and discussed latest urine sample report. Two crew members high:
A. Aman - 0.33
B. Booth - 0.33
Talked about application method and work procedures. Are following guidelines for procedure except have just begun using gloves (plastic) and new applicators.
F. Found new plastic pump (for 30 gal. drums) to be somewhat sloppy.
1. May take getting used to.
G. Found new plastic applicators have two problems:
1. Nozzle leaks on fingers and ground.
2. Had several pumps clog up due to crystalization of Silvisar 510.
H. Vern Melvin got quantity of chemical on hands maintaining mechanical parts of new applicators.

7/21/71 A. Nozzles of new applicators continue to drip and run back on crewmens' fingers.
B. No other reported or observed accidents.

7/22/71 A. No reported or observed accidents.
B. New applicators continue to leak more than old oil cans.

7/23/71 A. Leckner spilled a quantity of Silvisar on his shirt and side when lid of his container was left off.
B. No other reported or observed accidents.

7/26/71 A. No reported or observed accidents.

7/27/71 A. Lid of Silvisar container was loose and fell off when truck was moved. A large quantity of chemical spilled in the back of the truck. We stopped work and returned to the station where we washed out the truck and cleaned all equipment.

7/28/71 A. No reported or observed accidents.

7/29/71 A. No reported or observed accidents.

7/30/71 A. No reported or observed accidents.

8/2/71 A. Crew was taken out of the woods at noon after receiving most recent reports from Wenatchee and talking with Dr. Logan Norris. All crew members were reported as having a significant arsenic level in systems.

General Comments Concerning Work Procedure:

A. This is the second year I have run the crew. After our first report I ran a much cleaner operation than last year when our levels never attained such concentration.
B. Only change in crew procedure this year has been the preference by the crew to carry gallon containers (Purex bottles) with them to refill their applicators in the field.
C. Crew has not been using gloves when cleaning equipment at the end of the day.
D. The chemical all smelled the same to the crew. Some of the chemical seemed to crystalize more rapidly.
E. Crews greatest complaint was the odor caused by fumes.
F. We've been careful but not meticulous.

8/23/71
A. Crew went back to thinning after two weeks away from chemical.
B. Leckner had already terminated and returned to school.
C. McCrum missed the week, was on vacation.
D. Melvin worked the week thinning with remaining crew members. Came in closer contact with chemical than in previous weeks of thinning.
E. No reported or observed accidents with chemical.

8/24/71
A. Booth's termination day. Did not get urine sample.
B. Melvin took sick leave.
C. No reported or observed accidents.

8/25/71
A. Aman, Wallat, & Melvin only remaining crew members.
B. No reported or observed accidents with chemical.

8/26/71
A. Melvin worked with chemical half a day. Trapped in the afternoon.
B. No reported or observed accidents with chemical.

8/27/71
A. Crew worked half day thinning and half the day cleaning and readying equipment for storage.
B. Final day of thinning for summer crew.

Comments:

A. Training sessions on handling of chemical might prevent the problems we encountered this summer.
B. Crew members have questions which never received definite answers.

APPENDIX 8

Guide Lines for Chemical Thinning
Colville National Forest

1. All chemical thinning crew vehicles will have metal or plywood box with compartments and a lid with a hasp so that the box can be locked. This box will be used to keep the hatchets, applicators, gloves, towels, soap and chemical in.

2. MSMA or cacodylic acid may be used. The particular product will depend on the needs of the particular thinning job.

3. Chemical will be applied according to a manufacturer's specifications. Crew members will read and understand the label.

4. Handling of chemical containers, applicators, and hatchets will be done with gloves on.

5. No spillage of chemical will be tolerated.

6. A plastic pan will be used to set any container in when filling, to catch drips or spillage.

7. All hatchets will have the Ansul Company, (narrow bit). This bit can be welded to the Ansul Company hatchet head or can be welded to a 2 1/2 x 2 1/2 x 1 1/8 steel plate bored for 5/8" Stanley hammer handle. The handle will also be welded to the hatchet head. Narrow bits can be purchased from the T. S. . Company, P. O. Box 151, Flanders, New Jersey 07836.

8. All crew members will use oil can No. N. 25 Plews as an applicator. Applicator calibrated at 1 cc per pump.

9. Each crew member will carry on his belt a one-quart plastic container inserted into a canvas sheath. This container will be filled with chemical in the morning and at noon to refill the applicator.

10. When walking to and from the day's work all applicators will have a piece of 1/4" surgical tubing plugged with a metal screw on one end. The other end fitted over the end of the oil can spout to prevent any dripping of chemical on the forest floor.

11. All hatchets will be kept in a sheath, on a belt worn by each crew member, when not being used to make a frill.

12. During wet or cold weather neoprene gloves will be used. During hot, dry weather cotton gloves will be used and changed each day or at noon if excessive amounts of chemical are on the gloves. The dirty gloves will be put into a plastic bag and washed once a week for reuse.

13. The chops will be made horizontal on the stem and at least 2 feet off the ground, spaced evenly around the stem.

14. Chemical spilled on the clothes or gloves will be washed off immediately. Wash water will be disposed of by digging a hole and covering with soil. (At least 16 inches)

15. All contacts with the chemical (accidental spillage), will be reported to the foreman.

16. Hands will be washed with soap and water prior to smoking and eating, and at the end of the work day. Complete change of clothes at least twice a week. Crew members will bathe daily.

17. All paper towels used after washing will be placed in a plastic bag and returned to the station for disposal. At the Ranger station, they are disposed of by depositing in empty barrel.

18. When leaving the vehicle to go work all chemical containers will be in the vehicle with lids or caps on.

19. Applicators will be washed out after each day's work and filled with non-detergent oil to prevent corrosion. This oil can be re-used for approximately three weeks.

20. All residue from applicators and gallon containers having oil and MSMA mixtures will be brought back to the station and disposed of in a drum set up for this.

21. All chemicals will be moved from 30 gallon drums and five gallon containers with an all plastic hand pump. This can be purchased from Van Waters and Rogers Scientific Supply, 600 S. Spokane Street, Seattle, Washington.

22. Each foreman will keep a daily diary, recording any accidental spillage, by whom and why and any other problems that arise during the day. Also a general statement how the work progressed (quality job).

Guide Lines for Chemical Thinning
Siuslaw National Forest

1. Selection of crop or leave trees will be as in the standard Siuslaw thinning contract.

2. All chops to hold chemical will be made horizontal on the stem, at least 2 feet off of the ground, and spaced evenly around the stem. Chops on any one tree should be as close to the same height as opportunity allows.

3. DBH will be estimated and all chop made according to the following table:

DBH (Inches)	Even Spaced Chops	Squirts Per Chop	*Total C.C. Per Tree	C.C. per Inch DBH
1"	2	1/4	1.0	1.0
2"	2	1/3	2.0	1.0
3"	2	2/3	2.6	0.9
4"	2	1	4.0	1.0
5"	3	1	6.0	1.2
6"	3	1	6.0	1.0
7"	4	1	8.0	1.1
8"	4	1	8.0	1.0
9"	5	1	10.0	1.1
10"	5	1	10.0	1.0
11"	6	1	12.0	1.1
12"	6	1	12.0	1.0

* using 6400 Silva-Gun - 1.5 to 2.0 Cubic Centimeters (cc) per full squirt.

4. All chops will be made before applying chemical to stem. This will avoid contamination of skin from splashout - caused by chopping tree to which chemical has been previously applied.

5. All chopping tools will be, "Jim-Gem", safety brush axes - one hand model.
   Sold by: Forestry Suppliers, Inc.
   Box 8397
   Jackson, Miss. 39204

6. All applicators will use 6400 Silva-Guns and one quart plastic bottles. (24 mm cap) (with E.J. Lohmeyers's modified tip[1/]).
Sold by TSI Co., 205 Main Street, Matawan, New Jersey 07747.

7. Contact with the chemical will be avoided.
   A. Use protective equipment, such as face shields and rubber gloves when applying or handling chemical.
   B. Protect exposed skin with silicon base cream.
   C. Immediately wash off chemical spilled on skin, or wash out chemical spilled in eyes.
   D. Wash hands before eating and smoking.
   E. Change clothes and bathe daily.
   F. Change chemical soaked clothing immediately.
   G. Avoid skin contact with contaminated surfaces:
      1. When removing gloves, protective equipment, and clothing
      2. When handling application equipment,
      3. When filling applicator bottles.
   H. Wash off applicator and filling equipment after each days use, or if contaminated while filling, dry with paper towels.
   I. Store all chemical and equipment used with chemical in chemical locker each night.

8. All applicator equipment, bottles, funnels, jugs, filling equipment, etc., used with Silvicide will be marked with the name of the silvicide using a waterproof no-smear marker.

9. A plentiful supply of fresh wash water and soap (cold water no suds soap recommended) shall be on hand at the work site for crew members to wash, and to wash equipment, gloves, etc. Plenty of large size plastic dish pans should be available to hold wash water.

10. All wash watr, used paper towels, gloves, empty containers (perforated and mashed), etc.,shall be buried (daily or sooner) in a dirt pit at the work site. A minimum of one ft. of soil must cover residue in this pit.
    Dig the pit away from running or ground water and other potential contaminating spots. A dry, gentle sloped, well drained ridge is best.

11. Chemical spillage must be avoided. If accidental spillage should occur, bury the contaminated soil with other refuse.

12. The following protective equipment will be worn:
    1. Miranda or similar rubber gloves.
       Change when torn, or every 2 days.
    2. Rap-round face shield model 70-740 clear, 0.04 x 6" x 15".
       Bracket model 70-110 for cap and 70-112 for hat.
    3. Above items are available from:
       Sanderson Safety Supply Co.
       800 SE Hawthorne Blvd.
       Portland, Oregon 97214

1/ E. J. Lohmeyer, Waldport Ranger District, Siuslaw National Forest.
(503) 563-3211

13. Fill 1 qt. applicator bottles from modified 5 gallon plastic jug.[2/] Modificationconsists of a 1/2" diameter, 18" long P.V.C. plastic tube forced snugly over the jugs air vent hole. A cap from a 6400 Silva Gun is placed approximately 3" up on tube (Spray head has been broken out of cap). Tape is applied behind the cap to keep it from further sliding up the tube.
    1. 1/2" PVC tubing with 1/8" thick walls is available from:
       Shaw Surgical Co.
       1035 NW 14th Ave.
       Portland, Oregon 97208

14. Place snug fitting 1/2" tubing in top of bottle to be filled. Screw cap on tube loosely to b ttle to allow air to vent. Loosen main cap on Amitrol-T jug to allow air to vent. Tip jug until quart bottle has filled to 1 quart line. Tip jug back. Wait till most of the chemical has drained out of the tube. Remove one quart bottle. (It's a very clean fool-proof filling method). Fill over refuge pit or wash pan.

15. Fill 5 gal. Amitrol-T jug by using Angle stem no slash funnel available from Shaw Surgical Co. A funnel holder and stand may also prove useful.

2/ Details on modification available from Siuslaw National Forest.

Useful addresses:

1. Chemical handling equipment:
   Van Waters & Rogers
   3950 N.W.Yeon
   Portland, Oregon 97210

2. Safety equipment
   Sanderson Safety Supply Co.
   800 S.E.Hawthorne Blvd.
   Portland Oregon 97214

3. Chemical handling equipment and medical supplies:
   Shaw Surgical Co.
   902-912 S.W. Yamhill St.
   & 1035 N.W. 14th Ave.
   Portland Oregon 97208

4. Plastic tubing:
   M.L. Snyder & Co. Inc.
   2717 Western Ave.
   Seattle, Washington 98121

5. Jim-Gem brush axes:
   Forestry Suppliers, Inc.
   Box 8397
   Jackson, Mississippi 39204

6. 6400 Silva-Gun and quart plastic bottles:
   TSI Company
   205 Main St.
   Matawan, New Jersey 07747

7. 6400 Silva-Gun with 3ft. tubing:
   (GSA contract # GS-00S-88058)
   Afa Corp.
   Consumer Products Division
   P.O. Box 262
   Palatime, Illinois 60067

8. Plastic ware for science and industry:
   Bel-Art Products
   Pequannock, New Jersey 07440